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(54) Title: MULTIBINDING INHIBITORS OF TOPOISOMERASE

(57) Abstract

Disclosed are multibinding compounds which inhibit topoisomerases which are essential enzymes in higher encaryotes for DNA replication and repair. The multibinding compounds of this invention containing from 2 to 10 ligands covalently attached to one or more linkers. Each ligant is capable of binding to a topoisomerase and the distance between ligands is between about 2–50 Å. The multibinding compounds of this invention are useful in the treatment and prevention of cancer, microbial infections and the like.

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MULTIBINDING INHIBITORS OF TOPOISOMERASE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Patent Application Serial No. 60/088,448, filed June 8, 1998 and U.S. Patent Application Serial No. 60/093,072, filed July 16, 1998, the disclosures of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel multibinding compounds (agents) that inhibit topoisomerases which are essential enzymes in higher eucaryotes for DNA replication and repair and to pharmaceutical compositions comprising such compounds. Accordingly, the multibinding compounds and pharmaceutical compositions of this invention are useful in the treatment and prevention of cancer and microbial infections and the like.

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All of the above publications are herein incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

Topoisomerases are essential enzymes in higher eucaryotes for DNA replication and repair.[1,2,3,4] Antagonists of these enzymes have been prescribed both as antimicrobials and cancer chemotherapeutic agents. These antagonists have been used extensively in the treatment of cancer and are first-line

therapeutic agents (alone or in combination) for small-cell lung cancer, leukemias, lymphomas and germ cell tumors.[5,6,7]

Topoisomerase Mechanism of Action

The mammalian cell cycle consists of four distinct phases: M (mitosis); G_1 (gap 1, stage prior to DNA synthesis); S (DNA synthesis) and G_2 (gap 2, stage between DNA synthesis and mitosis). Topoisomerases function at G_1, G_2 and S phases of the cell cycle depending upon the topoisomerase isoform. Three mammalian forms of topoisomerases exist; Topo I, Topo IIα and Topo IIβ.[4]

Mammalian topoisomerase I (Topo I) is a 100 kD monomer acting at G_1 to relax supercoiled DNA prior to DNA synthesis.[5] The Topo I catalytic cycle is initiated by covalent binding of the N-terminal domain tyrosine to the 3' end of DNA. The C-terminal domain with its putative leucine zipper topology stabilizes the passage of the DNA. With each catalytic cycle, the DNA linking number increases by +1. Topo I causes transient ssDNA breaks independent of ATP hydrolysis.[2,3,4]

Mammalian Topo II, however, causes transient dsDNA breaks. Topo IIα and IIβ are approximately 180 kD proteins that form dimers *in situ*. Topo II is structurally similar to bacterial gyrase except that the gyrase forms tetramers *in situ*. Topo II's capacity to form dimers accounts for their ability to cause dsDNA breaks at S and G_2 phases of the cell cycle. The catalytic cycle proceeds as follows: An N-terminal domain tyrosine from each hemi-dimer covalently binds to the 5' portion of DNA (approximately four base pairs apart), dsDNA is cleaved, ATP is bound in the ATPase binding site of the enzyme and in a transesterification reaction, a second duplex is transported through the break and the strand religated. With ATP hydrolysis, the enzyme is recycled back to its original form.[1,2,3,4]

The regulation of the topoisomerases occurs at the level of transcription, translation and post-translationally. Upstream from the Topo I promoter are the consensus binding sequences for Sp1, NF-κB, cAMP-responsive element and members of the leucine-zipper protein motif.[8,9] Phorbol ester treatment induces a many-fold increase in Topo I transcription.[10] Transcriptional regulation of the Topo I gene is poorly understood and may depend upon the tumor type and the signaling pathway that predominates in that cell. In support of this theory is the discovery that p53 and *bcl*-2 influence gene transcription.[11,12]

Phosphorylation is the major post-translational modification of Topo I. Various protein kinases act upon the enzyme including Protein Kinase C (PKC) and casein kinase II.[13] Phosphorylation of the enzyme may regulate the activity of the enzyme. Tyrosine phosphorylation inactivates Topo I in vitro, serine phosphorylation enhances activity. Likewise, dephosphorylation by alkaline phosphatase inhibits the Topo I DNA relaxation activity and decreases sensitivity to the antagonist, camptothecin.[14] Therefore, phosphorylation may play a major role in enzyme activity, drug sensitivity and gene transcription.

Regulation of Topo II is also complex. Whereas Topo IIα mRNA levels fluctuate throughout the cell cycle, Topo IIβ mRNA levels remain constant. Likewise, in neoplasms, Topo IIα mRNA levels fluctuate and Topo IIβ mRNA levels remain constant. In line with these observations, the p53 protein negatively regulates expression of the Topo IIα gene.[15] Additional transcriptional consensus sequences include c-Myb, c-Myc, Sp-1, activating transcription factor (ATF) and NF-Y.[16,17,18] Those agents that induce cell differentiation; such as retinoids and phorbol esters, and, hence, cease cell replication decrease Topo IIα transcription.

Just as with Topo I, the predominant means of post-translational modification of Topo II is phosphorylation. A number of kinases phosphorylate

the enzyme including, casein kinase II, MAP kinase, cAMP-dependent protein kinase, PKC, stress-activated protein kinases (SAPK) and proline-directed kinases.[1,19,20] These kinases may play differing roles in the enzyme function. For example, the phosphorylation state of Topo II is changed throughout the cell cycle. Dephosphorylation of the enzyme inhibits the multimerization of the enzyme; casein kinase II restores dimerization.[21] The phosphorylation state may determine the enzyme's susceptibility to cytotoxic agents. Phosphorylation by casein kinase II or PKC decreases the sensitivity of Topo II to etoposide and tropetecan. The role of phosphorylation in drug resistance is unknown.

Inhibitors of Topoisomerases

Drugs that act on the topoisomerases can be divided into three broad classifications; intercalators, poisons and catalytic antagonists. Prior to enzyme antagonism, a topoisomerase-drug-DNA complex must be formed. There are three possible routes that this complex may be formed: 1) drug binding to DNA first; 2) drug binding to the enzyme-DNA complex or 3) drug bound to the enzyme first. In general, the intercalators bind to the DNA first and the catalytic antagonists and poisons bind either to the enzyme directly or after the DNA-enzyme complex has been formed.

Intercalators bind to the DNA directly and inhibit the interaction between the DNA and topoisomerases. Intercalators are non-specific inhibitors of both Topo I and Topo II. Examples of intercalators include the anthracyclines, mitoxantrone and actinomycin D.

Topoisomerase poisons are those compounds that increase the steady state concentration of the covalent DNA cleavage complexes. This interaction induces topoisomerase to act as a toxin that promotes DNA breaks without subsequent religation, causing mutagenic and lethal consequences to the cell. Topo I poisons include camptothecin and derivatives. Topo II poisons include etoposide,

ellipticine and derivatives. Poisons that are effective against both enzymes are saintopin and intoplicine. Quinolone derivatives may also be efficacious in cancer treatment.[5,22,23]

The DNA cleavage/religation equilibrium may be shifted dramatically dependent upon the cytotoxic agents utilized. For example, etoposide, tenopiside and amsacrine prevent the religation cycle whereas quinolones and intercalators are believed to promote DNA cleavage.[5,22]

Inhibition of topoisomerases causes numerous irreparable breaks in the DNA. Once these breaks are formed and the cell cannot recover, the cell enters a termination cycle known as programmed cell death (PCD) or apoptosis. Apoptosis is readily recognized on gels as multiple fragments of DNA (ladder formation).[24]

The catalytic inhibitors include suramin, novobiocin, chloroquine, fostriecin among others. These compounds tend to be poor substrates for the P-glycoprotein. The catalytic inhibitors are believed to act by inhibiting one of three ways: binding of the enzyme to DNA, prevention of ATP binding and inhibition of ATP hydrolysis. The catalytic inhibitors are relatively non-specific inhibitors of topoisomerase and the enzyme binding site is unknown. Catalytic inhibitors and poisons demonstrate antagonistic activity as one inhibits DNA binding and other prevents subsequent progression through the cleavage/religation cycle. In fact, it has been proposed that catalytic inhibitors be used as "rescuers" for normal tissues so that high-dose etoposide can be administered.[25,26]

Finally, inhibitors of Topo I and Topo II may antagonize the other's mechanism of action.[7] For example, etoposide may suppress camptothecin effects by inhibiting DNA replication and camptothecin may suppress the effects

of etoposide by inhibiting RNA transcription. As indicated above, catalytic inhibitors antagonize the efficacy of poisons by preventing the catalytic cycle. Cell cycle control may also play a pivotal role in the effectiveness of topoisomerase antagonists. If there is not sufficient time for DNA repair, defective DNA may accumulate, expediting the cell's demise.

Efficacy

In vitro efficacy is determined by inhibition of Topo II catalytic activity, namely Topo II decatenation reactions.[27] This reaction measures specific antagonism to Topo II. Briefly, kinetoplast DNA is mixed with purified Topo II and test compounds. After the incubation is complete, the products of the reaction are separated on a gel and analyzed for catenated DNA (antagonism), decatenated open circular (no drug effect) and decatenated closed circular DNA (no drug effect). Etoposide is effective in the μM range. To further study the mechanism of Topo II antagonism, Topo II cleavage reactions can be determined and DNA site-specificity identified. Likewise, religation reactions can be assessed by gel detection after Topo II-mediated religation in the presence of drug.[2,3,4,28]

In vivo efficacy is commonly assessed using the murine P388 leukemia model. More indicative of clinical efficacy, however, is testing a panel of human cancer xenografts in athymic mice. Progression of tumor, drug penetrance and animal survival can all be ascertained. Schedule-dependent antagonist activity can also be assessed in these models. Additionally, mRNA isolated from these tumors can be utilized to determine development of resistance through MDR or Topo II expression and Topo II mutatations.[29,30,31,32]

Resistance

Repeated exposure to cytotoxic drugs may induce resistance. In general, the resistance may be manifested by decreased expression of topoisomerase, mutations of the enzyme or via P-glycoprotein extrusion.[7]

Transcription can be potentially downregulated by a change in the inhibitory transcription factor expression, change in the upstream promoter region or the signaling pathways. In a mitoxantrone-treated cell line, there was a marked decrease in the Topo II mRNA levels and a truncated form of the enzyme was expressed.[5,6,33,34]

Although reduction of enzyme levels appears more common than mutations, gene rearrangement was demonstrated in a murine cell line exposed to adriamycin. Further, gene amplification and expression of two alleles of Topo IIα has been demonstrated. A mutant form of the enzyme in which the tyrosine binding site was replaced with a phenylalanine showed marked inhibition of etoposide binding. Resistance to catalytic inhibitors has been demonstrated and due to mutation of the ATPase domain.[7,33,34]

Hyperphosphorylation of the enzyme has been observed, however, it is unknown whether it is the result of a direct action of the chemotherapeutic agent or due to compensatory signaling pathways.[5,33,34]

P-glycoprotein actively removes many cytotoxic agents from the cell. With repeated administration, the MDR-1 gene is up regulated and an increased density of P-glycoprotein is expressed on the cell surface. Of the topoisomerase inhibitors, the following are substrates for P-glycoprotein: anthracyclines, topotecan, etoposide, mitoxantrone and amsacrine. Susceptibility of the anthracyclines to P-glycoprotein may be due to the amino group on daunosamine sugar. Camptothecin is not a substrate for P-glycoprotein.[5,7,33,34]

Toxicity

Because increased levels of topoisomerase activity are correlated with rapidly dividing cells, the most commonly recognized toxicities are associated with organs that have rapid cell turnover. Myelosuppression, alopecia and gastrointestinal toxicities are the most common manifestations of toxicity. Myelosuppression, because of the susceptibility to fatal infections, is the dose-limiting toxicity. Etoposide dosage of 100 mg/m^2/day for three days may induce severe leucopenia (< 1000 leucocytes/mm^3). However, a topotecan dosage of 2.7 mg/m^2/day for five days induced leucopenia whereas 4 mg/day was not associated with toxicity. Therefore, the dosing schedule may have a profound effect not only upon tumor regression but also upon toxicity.[35,36,37]

It has now been discovered that topoisomerase inhibitors having surprising and unexpected properties can be prepared by linking from 2 to 10 topoisomerase inhibitors to one or more linkers. Such multibinding compounds provide greater biological and/or therapeutic effects than the aggregate of the unlinked ligands due to their multibinding properties.

SUMMARY OF THE INVENTION

This invention is directed to novel multibinding compounds (agents) that inhibit topoisomerases. The multibinding compounds of this invention are useful in the treatment of cancer, microbial infection and the like.

Accordingly, in one of its composition aspects, this invention provides a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is between about 2 to 50Å; and pharmaceutically acceptable salts thereof.

In another of its composition aspects, this invention provides a multibinding compound of formula I:

$$(L)_p(X)_q \qquad I$$

wherein each L is independently a ligand capable of binding to a topoisomerase; each X is independently a linker; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; and further wherein the distance between ligands is about 2 to 50Å; and pharmaceutically acceptable salts thereof.

Preferably, *q* is less than *p* in the multibinding compounds of this invention.

In still another of its composition aspects, this invention provides a multibinding compound of formula II:

$$L'-X'-L' \qquad II$$

wherein each L' is independently a ligand capable of binding to a topoisomerase and X' is a linker; and further wherein the distance between the ligands is also about 2 to 50Å; and pharmaceutically- acceptable salts thereof.

Preferably, in the above embodiments, each linker (i.e., X, X' or X") independently has the formula:

$$-X^a-Z-(Y^a-Z)_m-Y^b-Z-X^a-$$

wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of

-O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -$S(O)_n$CR'R"-, -$S(O)_n$-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

In yet another of its composition aspects, this invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is about 2 to 50Å; and pharmaceutically acceptable salts thereof.

The multibinding compounds of this invention are effective inhibitors of the enzyme topoisomerase, an enzyme involved in DNA replication and repair. Accordingly, in one of its method aspects, this invention provides a method for treating cancer or microbial infections in a mammal comprising administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a multibinding compound comprising

from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is about 2 to 50Å; and pharmaceutically acceptable salts thereof.

This invention is also directed to general synthetic methods for generating large libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties for topoisomerase . The diverse multimeric compound libraries provided by this invention are synthesized by combining a library of linkers with a library of ligands each having complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity, polarizability and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

Additionally, this invention is directed to libraries of diverse multimeric compounds which multimeric compounds are candidates for possessing multibinding properties for topoisomerase. These libraries are prepared via the methods described above and permit the rapid and efficient evaluation of what molecular constraints impart multibinding properties to a ligand or a class of ligands for topoisomerase.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties for topoisomerase.

In another of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties for topoisomerase.

Preferably, in these methods, the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

Additionally, the multimeric ligand compounds comprising the multimeric ligand compound library are preferably dimeric. In one embodiment, the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric. The heterodimeric ligand compound library is preferably prepared by sequential addition of a first and second ligand.

In a preferred embodiment of the above methods, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library. More preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

In the above methods, the linker or linkers employed are preferably selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers. More preferably, the linkers comprise linkers of different chain length and/or having different complementary reactive groups. Still more preferably, the linkers are selected to have different linker lengths ranging from 2 to 50Å.

The ligand or mixture of ligands employed in the above methods is preferably selected to have reactive functionality at different sites on said ligands. More preferably, the reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive

functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In one preferred embodiment of the above methods, the multimeric ligand compound library comprises homomeric ligand compounds. In another preferred embodiment, the multimeric ligand compound library comprises heteromeric ligand compounds.

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for topoisomerase, which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may possess multivalent properties for topoisomerase, which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the linker or linkers employed are preferably selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers. More preferably, the linkers comprise linkers of different chain length and/or having different complementary reactive groups. Still more preferably, the linkers are selected to have different linker lengths ranging from about 2 to 50Å.

In the above libraries, the ligand or mixture of ligands is preferably selected to have reactive functionality at different sites on said ligands. Preferably, the reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In one embodiment, the multimeric ligand compound library comprises homomeric ligand compounds (i.e., each of the ligands is the same, although it

may be attached at different points). In another embodiment, the multimeric ligand compound library comprises heteromeric ligand compounds (i.e., at least one of the ligands is different from the other ligands).

In another of its method aspects, this invention is directed to an iterative method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties for topoisomerase;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties for topoisomerase;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated from 2-50 times. More preferably, steps (e) and (f) are repeated from 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates the structure of representative ligands.

FIG. 2 illustrates examples of multibinding compounds comprising 2 ligands attached in different formats to a linker.

FIG. 3 illustrates examples of multibinding compounds comprising 3 ligands attached in different formats to a linker.

FIG. 4 illustrates examples of multibinding compounds comprising 4 ligands attached in different formats to a linker.

FIG. 5 illustrates examples of multibinding compounds comprising >4 ligands attached in different formats to a linker.

FIG. 6 is the SAR for epipodophyllotoxin.

FIG. 7 illustrates dimerization orientations of epipodophyllotoxin derivatives.

FIG. 8 is the SAR for α - peltatin derivatives.

FIG. 9 illustrates dimerization of α - peltatin through phenol.

FIG. 10 is the SAR for azatoxin.

FIG. 11 illustrates indole linked azatoxin dimers.

FIG. 12 illustrates the synthesis of an intermediate that is used in preparing multibinding compounds.

FIGS. 13 and 14 illustrates structures of various starting compounds.

FIGS. 15-26 illustrate synthesis of multibinding compounds.

DETAILED DESCRIPTION OF THE INVENTION

This invention is directed to multibinding compounds which inhibit the enzyme topoisomerase, pharmaceutical compositions containing such compounds and methods for treating cancer and microbial infections in mammals. The focus of the specification will be on multibinding compounds for treating cancer but it is understood that other indications are included as well. When discussing such compounds, compositions or methods, the following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *iso*-butyl, *n*-hexyl, *n*-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene ($-CH_2-$), ethylene ($-CH_2CH_2-$), the propylene isomers (e.g., $-CH_2CH_2CH_2-$ and $-CH(CH_3)CH_2-$) and the like.

The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl. Additionally, such substituted alkylene groups include those

where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused groups contain from 1 to 3 fused ring structures.

The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy ($-CH_2OCH_3$), ethylenemethoxy ($-CH_2CH_2OCH_3$), *n*-propylene-*iso*-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-*t*-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ($-CH_2SCH_3$), ethylenethiomethoxy ($-CH_2CH_2SCH_3$), *n*-propylene-*iso*-thiopropoxy ($-CH_2CH_2CH_2SCH(CH_3)_2$), methylene-*t*-thiobutoxy ($-CH_2SC(CH_3)_3$) and the like.

The term "alkenyl" refers to a monoradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred alkenyl groups include ethenyl ($-CH=CH_2$), *n*-propenyl ($-CH_2CH=CH_2$), *iso*-propenyl ($-C(CH_3)=CH_2$), and the like.

The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkenylene" refers to a diradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon

atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., $-CH_2CH=CH-$ and $-C(CH_3)=CH-$) and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl groups include ethynyl (-C≡CH), propargyl ($-CH_2C\equiv CH$) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido,

cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynylene groups include ethynylene (-C≡C-), propargylene ($-CH_2C\equiv C-$) and the like.

The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl

The term "acyl" refers to the groups HC(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic-C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl,

cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acylamino" or "aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" or "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to the diradical derived from aryl (including substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH_2.

The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" or "alkoxycarbonyl" refers to the groups "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy,

halo, cyano, nitro, trihalomethyl, and thioalkoxy. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

The term "heteroarylene" refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridnylene, 2,5-indolenyl and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "spiro-attached cycloalkyl group" refers to a cycloalkyl group attached to another ring via one carbon atom common to both rings.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically-acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically-acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines, tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines,

substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid,

maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, *p*-toluene-sulfonic acid, salicylic acid, and the like.

The term "pharmaceutically-acceptable cation" refers to the cation of a pharmaceutically-acceptable salt.

The term "protecting group" or "blocking group" refers to any group which when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the compounds (including intermediates thereof) prevents reactions from occurring at these groups and which protecting group can be removed by conventional chemical or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group. The particular removable blocking group employed is not critical and preferred removable hydroxyl blocking groups include conventional substituents such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-butyl-diphenylsilyl and any other group that can be introduced chemically onto a hydroxyl functionality and later selectively removed either by chemical or enzymatic methods in mild conditions compatible with the nature of the product.

Preferred removable thiol blocking groups include disulfide groups, acyl groups, benzyl groups, and the like.

Preferred removable amino blocking groups include conventional substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), fluorenylmethoxycarbonyl (FMOC), allyloxycarbonyl (ALOC), and the like which can be removed by conventional conditions compatible with the nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl, propyl, *t*-butyl etc. which can be removed by mild conditions compatible with the nature of the product.

The term "optional" or "optionally" means that the subsequently described event, circumstance or substituent may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

The term "ligand" as used herein denotes a compound that binds to the enzyme topoisomerase. The specific region or regions of the ligand that is (are) recognized by the enzyme or enzyme-DNA complex is designated as the "ligand domain". A ligand may be either capable of binding to an enzyme (or enzyme-DNA complex or initially the DNA prior to forming the complex) by itself, or may require the presence of one or more non-ligand components for binding (e.g., Ca^{+2}, Mg^{+2} or a water molecule is required for the binding of a ligand to various ligand binding sites).

Examples of ligands useful in this invention include, for example, topoisomerase II inhibitors such as A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, ellipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone,

pyridobenzophenoxazine, S-16020-2, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and analogues thereof.

Topoisomerase I inhibitors include, for example, camptothecin, saintopin (which is also a topoisomerase II inhibitor) and analogues thereof.

The structures of selected representative ligands are illustrated in Figure 1.

Those skilled in the art will appreciate that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced or substituted with unrelated structures (for example, with ancillary groups as defined below) and, in some cases, omitted entirely without affecting the binding interaction. The primary requirement for a ligand is that it has a ligand domain as defined above. It is understood that the term ligand is not intended to be limited to compounds known to be useful in binding to topoisomerase (e.g., known drugs). Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally associated with enzyme binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multivalent compounds because of the benefits conferred by multivalency.

The term "multibinding compound or agent" refers to a compound that is capable of multivalency, as defined below, and which has 2-10 ligands covalently bound to one or more linkers which may be the same or different. Multibinding compounds provide a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which are made available for

binding. That is to say that the biological and/or therapeutic effect of the ligands attached to the multibinding compound is greater than that achieved by the same amount of unlinked ligands made available for binding to the ligand binding sites (receptors). The phrase "increased biological or therapeutic effect" includes, for example: increased affinity, increased selectivity for target, increased specificity for target, increased potency, increased efficacy, decreased toxicity, improved duration of activity or action, increased ability to kill cells such as fungal pathogens, cancer cells, etc., decreased side effects, increased therapeutic index, improved bioavailibity, improved pharmacokinetics, improved activity spectrum, and the like. The multibinding compounds of this invention will exhibit at least one and preferably more than one of the above-mentioned affects.

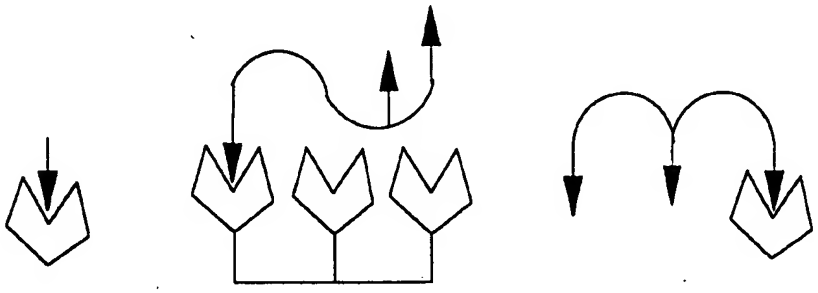
Dimers of etoposide have been synthesized by reacting the 4'-phenol with diacids (e.g. sebacic, adipic acids)[39]. These dimers, in common with other 4'-phenol acylation products, do not show topoisomerase activity. Therefore, multibinding compounds of the present invention do not include these non-active dimers.

The term "multimeric compound" refers to a compound containing 2 to 10 ligands covalently connected through at least one linker which compound may or may not possess multibinding properties.

The term "potency" refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases, the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multibinding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g., in an *in vitro* or *in vivo* assay, in an appropriate animal model.) The finding that the multbinding agent produces an equivalent

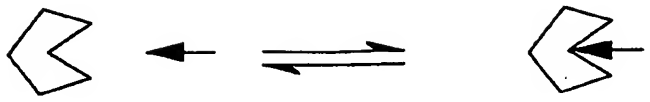
biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand is indicative of enhanced potency.

The term "univalency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It should be noted that a compound having multiple copies of a ligand (or ligands) exhibit univalency when only one ligand is interacting with a ligand binding site. Examples of univalent interactions are depicted below.

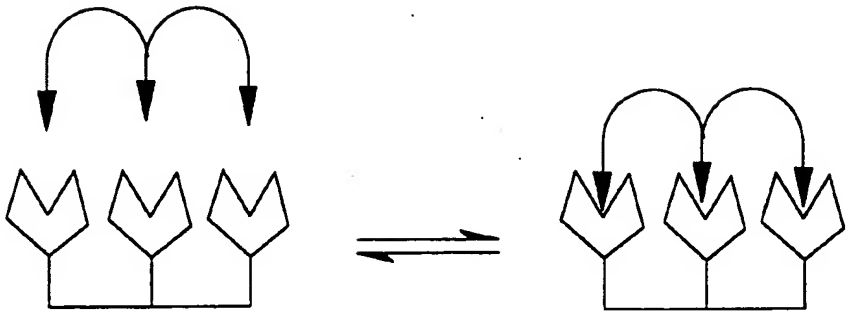


The term "multivalency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding receptors (ligand binding sites) on one or more enzymes which may be the same or different.

For example, two ligands connected through a linker that bind concurrently to two ligand binding sites would be considered as bivalency; three ligands thus connected would be an example of trivalency. An example of trivalent binding, illustrating a multibinding compound bearing three ligands versus a monovalent binding interaction, is shown below:



Univalent Interaction



Trivalent Interaction

It should be understood that all compounds that contain multiple copies of a ligand attached to a linker or to linkers do not necessarily exhibit the phenomena of multivalency, i.e., that the biological and/or therapeutic effect of the multibinding agent is greater than the sum of the aggregate of unlinked ligands made available for binding to the ligand binding site (receptor). For multivalency to occur, the ligands that are connected by a linker or linkers have to be presented to their ligand binding sites by the linker(s) in a specific manner in order to bring about the desired ligand-orienting result, and thus produce a multibinding event.

The term "selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites (receptors). The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or, in cases

where a biological effect is observed below the K_d , the ratio of the respective EC_{50}'s (i.e., the concentrations that produce 50% of the maximum response for the ligand interacting with the two distinct ligand binding sites (receptors)).

The term "ligand binding site" denotes the site on the topoisomerase enzyme (or enzyme-DNA complex or initially the DNA prior to forming the complex) that recognizes a ligand domain and provides a binding partner for the ligand. The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example, agonism, antagonism, modulatory effects, may maintain an ongoing biological event, and the like.

It should be recognized that the ligand binding sites of the enzyme that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and inter-molecular associations (e.g., such macromolecular structures may be covalently joined to a single structure, noncovalently associated in a multimeric structure, embedded in a membrane or polymeric matrix, and so on) and therefore have less translational and rotational freedom than if the same structures were present as monomers in solution.

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.

The term "inert organic solvent" or "inert solvent" means a solvent which is inert under the conditions of the reaction being described in conjunction therewith including, by way of example only, benzene, toluene, acetonitrile, tetrahydrofuran, dimethylformamide, chloroform, methylene chloride, diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol,

isopropanol, *t*-butanol, dioxane, pyridine, and the like. Unless specified to the contrary, the solvents used in the reactions described herein are inert solvents.

The term "treatment" refers to any treatment of a pathologic condition in a mammal, particularly a human, and includes:

(i) preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition;

(ii) inhibiting the pathologic condition, i.e., arresting its development;

(iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or

(iv) relieving the conditions mediated by the pathologic condition.

The term "pathologic condition which is modulated by treatment with a ligand" covers all disease states (i.e., pathologic conditions) which are generally acknowledged in the art to be usefully treated with a ligand for the enzyme topoisomerase in general, and those disease states which have been found to be usefully treated by a specific multibinding compound of our invention. Such disease states include, by way of example only, the treatment of a mammal afflicted with cancer, microbial infection, and the like.

The term "therapeutically effective amount" refers to that amount of multibinding compound which is sufficient to effect treatment, as defined above, when administered to a mammal in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "linker" or "linkers" as used herein, identified where appropriate by the symbol X, X' or X", refers to a group or groups that covalently link(s) from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multivalency. Each linker may be chiral or achiral. Among other features, the linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. In some cases, the linker may itself be biologically active. The term "linker" does not, however, extend to cover solid inert supports such as beads, glass particles, fibers, and the like. But it is understood that the multibinding compounds of this invention can be attached to a solid support if desired. For example, such attachment to solid supports can be made for use in separation and purification processes and similar applications.

The ligands and linkers which comprise the multibinding agents of the invention and the multibinding compounds themselves may have various stereoisomeric forms, including enantiomers and diastereomers. It is to be understood that the invention contemplates all possible stereoisomeric forms of multibinding compounds, and mixtures thereof.

The extent to which multivalent binding is realized depends upon the efficiency with which the linker or linkers that joins the ligands presents these ligands to the array of available ligand binding sites. Beyond presenting these ligands for multivalent interactions with ligand binding sites, the linker or linkers spatially constrains these interactions to occur within dimensions defined by the linker or linkers. Thus, the structural features of the linker (valency, geometry, orientation, size, flexibility, chemical composition, etc.) are features of multibinding agents that play an important role in determining their activities.

The linkers used in this invention are selected to allow multivalent binding of ligands to the ligand binding sites of topoisomerase (or enzyme-DNA complex

or initially the DNA prior to forming the complex), whether such sites are located interiorly, both interiorly and on the periphery of the enzyme structure, or at any intermediate position thereof. The distance between the nearest neighboring ligand domains is preferably between about 2 to 50Å, more preferably between about 10Å to about 30Å and most preferably between about 14Å to about 27Å.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the linker or linkers. Reaction chemistries resulting in such linkages are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for bonding or which can be introduced onto the ligand for bonding. Again, such complementary functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable, well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

Table I below illustrates numerous complementary reactive groups and the resulting bonds formed by reaction there between.

Table I

Representative Complementary Binding Chemistries

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-hydroxyamine
sulfonyl halide	amine	sulfonamide
carboxyl	amine	amide
hydroxyl	alkyl/aryl halide	ether

The linker is attached to the ligand at a position that retains ligand domain-ligand binding site interaction and specifically which permits the ligand domain of the ligand to orient itself to bind to the ligand binding site. Such positions and synthetic protocols for linkage are well known in the art. The term linker embraces everything that is not considered to be part of the ligand.

The relative orientation in which the ligand domains are displayed derives from the particular point or points of attachment of the ligands to the linker, and on the framework geometry. The determination of where acceptable substitutions can be made on a ligand is typically based on prior knowledge of structure-activity relationships (SAR) of the ligand and/or congeners and/or structural information about ligand-receptor complexes (e.g., X-ray crystallography, NMR, and the like). Such positions and the synthetic methods for covalent attachment are well known in the art. Following attachment to the selected linker (or attachment to a significant portion of the linker, for example 2-10 atoms of the linker), the univalent linker-ligand conjugate may be tested for retention of activity in the relevant assay.

Suitable linkers are discussed more fully below.

At present, it is preferred that the multibinding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X.

The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits the facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

The term "pseudohalide" refers to a functional group which react in a displacement reaction in a manner similar to a halogen, e.g., functions as a leaving group is a displacement reaction. Such functional groups include, by way of example, mesyl, tosyl, azido, cyano and the like.

Methodology

The linker, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multibinding compound. The biological activity of the multibinding compound is highly sensitive to the valency, geometry, composition, size, flexibility or rigidity, etc. of the linker and, in turn, on the overall structure of the multibinding compound, as well as the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity of the linker, and the like on the linker.

Accordingly, the linker is preferably chosen to maximize the biological activity of the multibinding compound. The linker may be chosen to enhance the biological activity of the molecule. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands to their ligand binding sites to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring about the desired ligand-orienting result, and thus produce a multibinding compound.

For example, different orientations can be achieved by including in the framework groups containing mono- or polycyclic groups, including aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight-chain species. In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In other preferred embodiments, the ring is a six or ten member ring. In still further preferred embodiments, the ring is an aromatic ring such as, for example, phenyl or naphthyl.

Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moieties can readily be controlled by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ($H_2N(CH_2)_6NH_2$) or related polyamines can be modified to be substantially more hydrophilic by replacing the alkylene group with a poly(oxyalkylene) group such as found in the commercially available "Jeffamines".

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns.

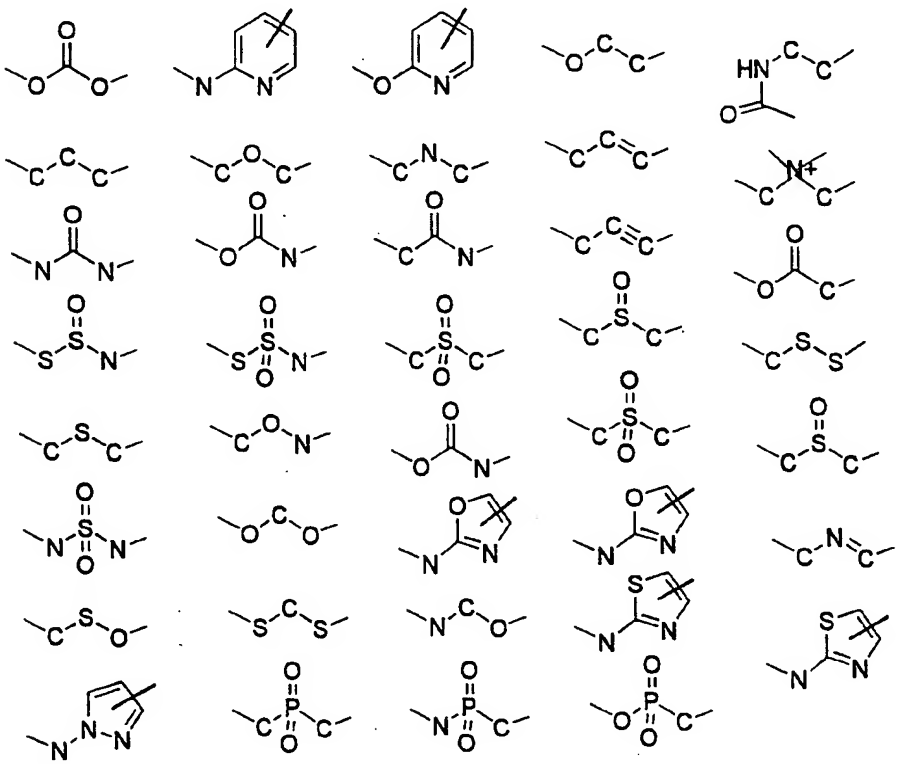
Examples of acceptable patterns of three contiguous atom arrangements are shown in the following diagram:

	CCC	NCC	OCC	SCC	PCC
	CCN	NCN	OCN	SCN	PCN
	CCO	NCO	OCO	SCO	PCO
	CCS	NCS	OCS	SCS	PCS
	CCP	NCP	OCP	SCP	PCP
	CNC	NNC	ONC	SNC	PNC
	CNN	NNN	ONN	SNN	PNN
	CNO	NNO	ONO	SNO	PNO
	CNS	NNS	ONS	SNS	PNS
	CNP	NNP	ONP	SNP	PNP
	COC	NOC	OOC	SOC	POC
	CON	NON	OON	SON	PON
	COO	NOO	OOO	SOO	POO
	COS	NOS	OOS	SOS	POS
	COP	NOP	OOP	SOP	POP
	CSC	NSC	OSC	SSC	PSC
	CSN	NSN	OSN	SSN	PSN
	CSO	NSO	OSO	SSO	PSO
	CSS	NSS	OSS	SSS	PSS
	CSP	NSP	OSP	SSP	PSP
	CPC	NPC	OPC	SPC	PPC
	CPN	NPN	OPN	SPN	PPN
	CPO	NPO	OPO	SPO	PPO

CPS	NPS	OPS	SPS	PPS
CPP	NPP	OPP	SPP	PPP

One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March, "Advanced Organic Chemistry", 4th Edition, Wiley-Interscience, New York, New York (1992). These arrangements are described in the grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.



The identification of an appropriate framework geometry and size for ligand domain presentation are important steps in the construction of a multibinding compound with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process. Figure 2 illustrates a useful strategy for determining an optimal framework display orientation for ligand domains. Various other strategies are known to those skilled in the art of molecular design and can be used for preparing compounds of this invention.

As shown in Figure 2, display vectors around similar central core structures such as a phenyl structure and a cyclohexane structure can be varied, as can the spacing of the ligand domain from the core structure (i.e., the length of the attaching moiety). It is to be noted that core structures other than those shown here can be used for determining the optimal framework display orientation of the ligands. The process may require the use of multiple copies of the same central core structure or combinations of different types of display cores.

The above-described process can be extended to trimers (Figure 3) and compound of higher valency (Figures 4 and 5).

Assays of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g., potency, selectivity, etc.). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. A wide diversity of linkers is commercially available (see, e.g., Available Chemical Directory (ACD)). Many of the linkers that are suitable for use in this invention fall into this category.

Other can be readily synthesized by methods well known in the art and/or are described below.

Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition thereof. The composition of the linker can be varied in numerous ways to achieve the desired physical properties for the multibinding compound.

It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic or anionic groups, or a combination thereof.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into or onto the linker, for example, to change the solubility of the multibinding compound (in water, fats, lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto or into the linker enhances the hydrophilicity and water solubility of the multibinding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG may decrease antigenicity and potentially enhances the overall rigidity of the linker.

Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multibinding compounds are useful in practicing this invention. Thus, it is within the scope of the present invention to

use ancillary groups such as, for example, small repeating units of ethylene glycols, alcohols, polyols (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligosaccharides, etc.), carboxylates (e.g., small repeating units of glutamic acid, acrylic acid, etc.), amines (e.g., tetraethylenepentamine), and the like) to enhance the water solubility and/or hydrophilicity of the multibinding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether .

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multibinding compounds described herein is also within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above, may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of this invention is the use of ancillary groups which result in the multibinding compound being incorporated or anchored into a vesicle or other membranous structure such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer or a micelle such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are

phosphglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds within the group, for example, aryl, heteroaryl, cycloalkyl, cycloalkenyl, and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will

tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following addition to the linker, include deprotectation of a carboxyl, hydroxyl, thiol or amino group by a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art which result in removal of the protecting group, is within the scope of this invention.

Rigidity may also be imparted by internal hydrogen bonding or by hydrophobic collapse.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In other preferred embodiments, the linker comprises one or more six-membered rings. In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, restricted/unrestricted rotation, the desired degree of hydrophobicity/hydrophilicity, etc. is well within the skill of the art. Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention. In certain cases, the

antigenicity of a multibinding compound may be eliminated or reduced by use of groups such as, for example, poly(ethylene glycol).

As explained above, the multibinding compounds described herein comprise 2-10 ligands attached to a linker that links the ligands in such a manner that they are presented to the enzyme for multivalent interactions with ligand binding sites thereon/therein. The linker spatially constrains these interactions to occur within dimensions defined by the linker. This and other factors increases the biological activity of the multibinding compound as compared to the same number of ligands made available in monobinding form.

The compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is described below.

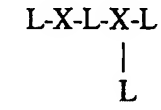
As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

The simplest and most preferred multibinding compound is a bivalent compound which can be represented as L-X-L, where each L is independently a ligand which may be the same or different and each X is independently the linker. Examples of such bivalent compounds are provided in FIG. 2 where each shaded circle represents a ligand. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X. However, a trimer can also be a radial multibinding compound comprising three

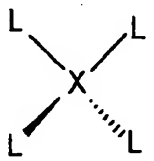
ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Illustrations of trivalent and tetravalent compounds of this invention are found in FIG.s 3 and 4 respectively where, again, the shaded circles represent ligands. Tetravalent compounds can be represented in a linear array, e.g.,

L-X-L-X-L-X-L

in a branched array, e.g.,



(a branched construct analogous to the isomers of butane -- *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl) or in a tetrahedral array, e.g.,

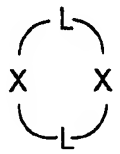


where X and L are as defined herein. Alternatively, it could be represented as an alkyl, aryl or cycloalkyl derivative as above with four (4) ligands attached to the core linker.

The same considerations apply to higher multibinding compounds of this invention containing 5-10 ligands as illustrated in FIG. 5 where, as before, the shaded circles represent ligands. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint that

there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not directly accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

Certain of the above described compounds may alternatively be represented as cyclic chains of the form:



and variants thereof.

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

With the foregoing in mind, a preferred linker may be represented by the following formula:

$$-X^a\text{-}Z\text{-}(Y^a\text{-}Z)_m\text{-}Y^b\text{-}Z\text{-}X^a\text{-}$$

in which:

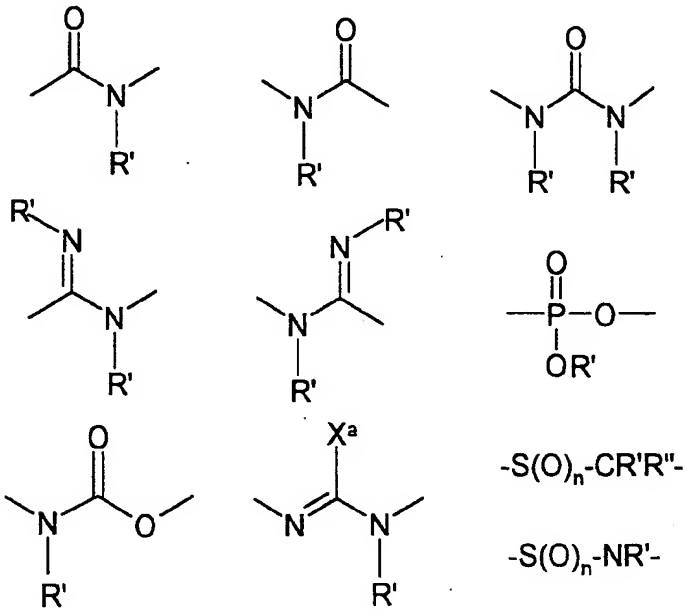
m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene,

cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of:



-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

In one embodiment of this invention, the linker (i.e., X, X' or X") is selected those shown in Table II:

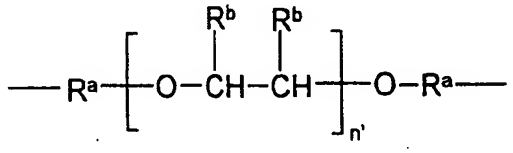
Table II

Representative Linkers

Linker
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_4$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_5$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_6$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_7$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_8$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_9$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{10}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{11}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_{12}$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,2-phenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-Z-O-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-$(CH_2)_8$-CH_3)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-O-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-(*n*-octadecyloxy)-1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Z)-C(O)-NH-$(CH_2)_2$-NH- where Z is 4-biphenyl

Linker
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-(n-butyloxy)-1,3-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_8$-*trans*-(CH=CH)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-$(CH_2)_{12}$-CH_3)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Z)-C(O)-NH-$(CH_2)_2$-NH- where Z is 4-(n-octyl)-phenyl
-HN-(CH_2)-Z-O-$(CH_2)_6$-O-Z-(CH_2)-NH- where Z is 1,4-phenyl
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-CH(NH-C(O)-Ph)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-N+(($(CH_2)_9$-CH_3)(CH_2-C(O)-NH-$(CH_2)_2$-NH_2)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-(CH_2)-N(($(CH_2)_9$-CH_3)-(CH_2)-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_2$-NH-C(O)-$(CH_2)_3$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-C(O)-NH-$(CH_2)_2$-NH-
-HN-$(CH_2)_2$-NH-C(O)-Z-C(O)-NH-$(CH_2)_2$-NH- where Z is 5-hydroxy-1,3-phenyl

In another embodiment of this invention, the linker (i.e., X, X' or X") has the formula:



wherein

each R^a is independently selected from the group consisting of a covalent bond, alkylene, substituted alkylene and arylene;

each R^b is independently selected from the group consisting of hydrogen, alkyl and substituted alkyl; and

n' is an integer ranging from 1 to about 20.

In yet another embodiment, the linker (i.e., X or X') has the formula: $-(CH_2)_{n'}-$, where *n'* is an integer ranging from 1 to about 20, preferably from 2 to 6.

In view of the above description of the linker, it is understood that the term "linker" when used in combination with the term "multibinding compound" includes both a covalently contiguous single linker (e.g., L-X-L) and multiple covalently non-contiguous linkers (L-X-L-X-L) within the multibinding compound.

Preparation of Multibinding Compounds

The multibinding compounds of this invention can be prepared from readily available starting materials using the following general methods and procedures. It will be appreciated that where typical or preferred process conditions (i.e., reaction temperatures, times, mole ratios of reactants, solvents, pressures, etc.) are given, other process conditions can also be used unless otherwise stated. Optimum reaction conditions may vary with the particular reactants or solvent used, but such conditions can be determined by one skilled in the art by routine optimization procedures.

Additionally, as will be apparent to those skilled in the art, conventional protecting groups may be necessary to prevent certain functional groups from undergoing undesired reactions. The choice of a suitable protecting group for a particular functional group as well as suitable conditions for protection and deprotection are well known in the art. For example, numerous protecting groups, and their introduction and removal, are described in T. W. Greene and

G. M. Wuts, *Protecting Groups in Organic Synthesis*, Second Edition, Wiley, New York, 1991, and references cited therein.

Any compound which inhibits topoisomerase can be used as a ligand in this invention. As discussed in further detail below, numerous such topoisomerase inhibitors are known in the art and any of these known compounds or derivatives thereof may be employed as ligands in this invention. Typically, a compound selected for use as a ligand will have at least one functional group, such as an amino, hydroxyl, thiol or carboxyl group and the like, which allows the compound to be readily coupled to the linker. Compounds having such functionality are either known in the art or can be prepared by routine modification of known compounds using conventional reagents and procedures.

The ligand can be covalently attached to the linker through any available position on the ligand, provided that when the ligand is attached to the linker, the ligand retains its ability to bind to topoisomerase.

Because of topoisomerase II's dimeric structure, dimerization of topoisomerase II enzyme antagonists is a preferred approach. The following describes representative topoisomerase II ligands, namely, epipodophyllotoxin, α-peltatin, and azatoxin derivatives.

Epipodophyllotoxin Derivatives

Many modifications of podophyllotoxin have been made. The Structure Activity Relationships (SAR) with respect to topoisomerase inhibition, though, is less clear. The reason is that topoisomerase inhibition was not known to be the mechanism of action of etoposide prior to 1982[38]. For example, podophyllotoxin itself is an active cytotoxic agent, yet does not inhibit topoisomerase II, whereas the aglycone of etoposide is both cytotoxic and inhibits topoisomerase II. The

SAR of podophyllotoxin derivatives with respect to topoisomerase inhibition is shown in Figure 6 and summarized as follows:

2,3-positions: A *trans* fused ring is important. Both lactones and lactams show topoisomerase II inhibition, but the lactone was more cytotoxic[39].

4-position: A wide range of derivatives, with and without sugars, both hydrophobic and hydrophilic, have been synthesized and tested. Some of the more potent ones contain anilines at this position[40]. Esters, ethers, thioethers and amines have also been made and shown to be potent[41]. This is the position where the most synthetic activity has been directed. The stereochemisty here is important - the epipodophyllotoxin derivatives being superior to their epimers.

6,7-positions: Replacement of the methylenedioxy moiety led to an approximately 3-fold drop in activity. Groups used here include bis-phenol, bis-acetate, bis-methoxy and thiocarbonate[39].

2'-position: Functionalization here results in loss of activity[39].

3'-position: Removal of the methyl group (giving a catechol derivative) retains topoisomerase II potency. Oxidation to the ortho-quinone gives an equal or more potent compound. No introduction of larger alkyl groups has been performed[42].

4'-position: The phenol is critical for topoisomerase II activity. Alkylation removes activity. This position has been used for pro-drug attachment (etopophos).

Dimer Synthesis

Since it is unclear how these compounds bind the most promising orientations must be determined experimentally. Dimerization can be envisaged to proceed through at least three orientations which are illustrated in Figure 7:

The 4-position. The 4-bromoalkane can be treated with nucleophiles to produce substituted epipodophyllotoxin derivatives. Either bis-nucleophiles can be used directly (eg diamino alkanes) or some form of functionalization followed by dimerization could occur.

The 6,7-positions. The methylenedioxy group can be removed leaving a catechol. Reaction with an aldehyde would give a cyclic functionalized derivative. Either direct dimerization using a bis-aldehyde or reaction with a mono-aldehyde followed by dimerization could be used. Furthermore alkylation of one phenol could be achieved with alkyl halides and base.

The 3'-position. Alkylation of the phenol (after removal of the methyl group) with alkyl halides (in the presence of base) could lead to active dimers.

<u>α-Peltatin Derivatives</u>

α-Peltatin is a close relative of podophyllotoxin and also shows cytotoxic properties. It has a lower activity against topoisomerase II than etoposide and has been less studied. The SAR is shown in Figure 8. As can be seen, the SAR is similar to the epipodophyllotoxins, with the exception being loss of the methylenedioxy to give a catechol resulted in a more potent compound. The 5-phenol has been alkylated and acylated, often giving more potent compounds[41].

Dimer Synthesis

A preferred method is to dimerize through the 5-phenol which is shown in Figure 9. This gives products with a slightly different orientation than those

available with the epipodophyllotoxins. Dimerization through the 6,7-positions and the 3'-position could also be achieved.

Azatoxin Derivatives

Azatoxin (Figure 13, when R=H) is a synthetic epipdodophyllotoxin relative; it has equivalent topoisomerase II activity. An indole replaces the A and B rings and a cyclic urethane replaces the lactone. Several derivatives have been synthesized[43]. The SAR is shown in Figure 10.

Dimer Synthesis.

Azatoxin, could be dimerized through similar orientations to that of the 4 and 3'-positions in epipodophyllotoxin, or could be dimerized through several positions on the indole. Since the starting material for azatoxin synthesis is tryptophanol (2 steps, 90% yield) one could start with a suitably substituted tryptophan to generate a handle for dimerization. Commercially available examples include 5-hydroxy or 5-bromo tryptophan and 7-benzyloxytryptophan. Substitution in the 4 and 6-positions on the indole can be achieved by nitration. Furthermore substitution on the indole nitrogen can be achieved by alkylation. This chemistry is summarized in Figure 11.

Combinatorial Libraries

The methods described herein lend themselves to combinatorial approaches for identifying multimeric compounds which possess multibinding properties.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of

candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

Selection of Ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets, i.e., topoisomerase. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility, logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not sufficiently potent at a particular target so as to be efficacious in

a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

Orientation: Selection of Ligand Attachment Points and Linking Chemistry

Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the effects of presenting the ligands to their target binding site(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a ligand bound to its target allows one to identify one or more sites where linker attachment will not preclude the ligand/target interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643, the disclosure of which is incorporated herein by reference in its entirety. When such structural information is not available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple distinct orientations.

Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a ligand bound to its target, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to interact with the same target at sites proximal to the first binding site, which include elements of the target that are not part of the formal ligand binding site and/or elements of the matrix surrounding the formal binding site, such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule may be achieved by attaching it to the linker at a position which abrogates activity of the ligand at the first binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets.

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred

types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically innocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linker Selection

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency: In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore, divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker Length: Linkers are chosen in a range of lengths to allow the spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets. In other instances where high-resolution structural information is not available, one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are about 2-50Å, more preferably between about 10-30Å and most preferably between about 14-27Å.

Linker Geometry and Rigidity: The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two ligands to the ortho, meta, and para positions of a benzene ring, or in *cis*- or *trans*-arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis*- or *trans*-arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

Linker Physical Properties: The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties (hydrophobicity, hydrophilicity, amphiphilicity, polarization, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

Linker Chemical Functional Groups: Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

Having chosen a set of *n* ligands (*n* being determined by the sum of the number of different attachment points for each ligand chosen) and *m* linkers by the process outlined above, a library of (*n*!)*m* candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalies on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial libary is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values are determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, ion channel blockers, and antimicrobial activity, are also determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data are determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application

Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libraries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand orientations, linker lengths, linker geometries, etc. Additional libraries are then generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

To further elaborate upon this procedure, suitable divalent linkers include, by way of example only, those derived from dicarboxylic acids,

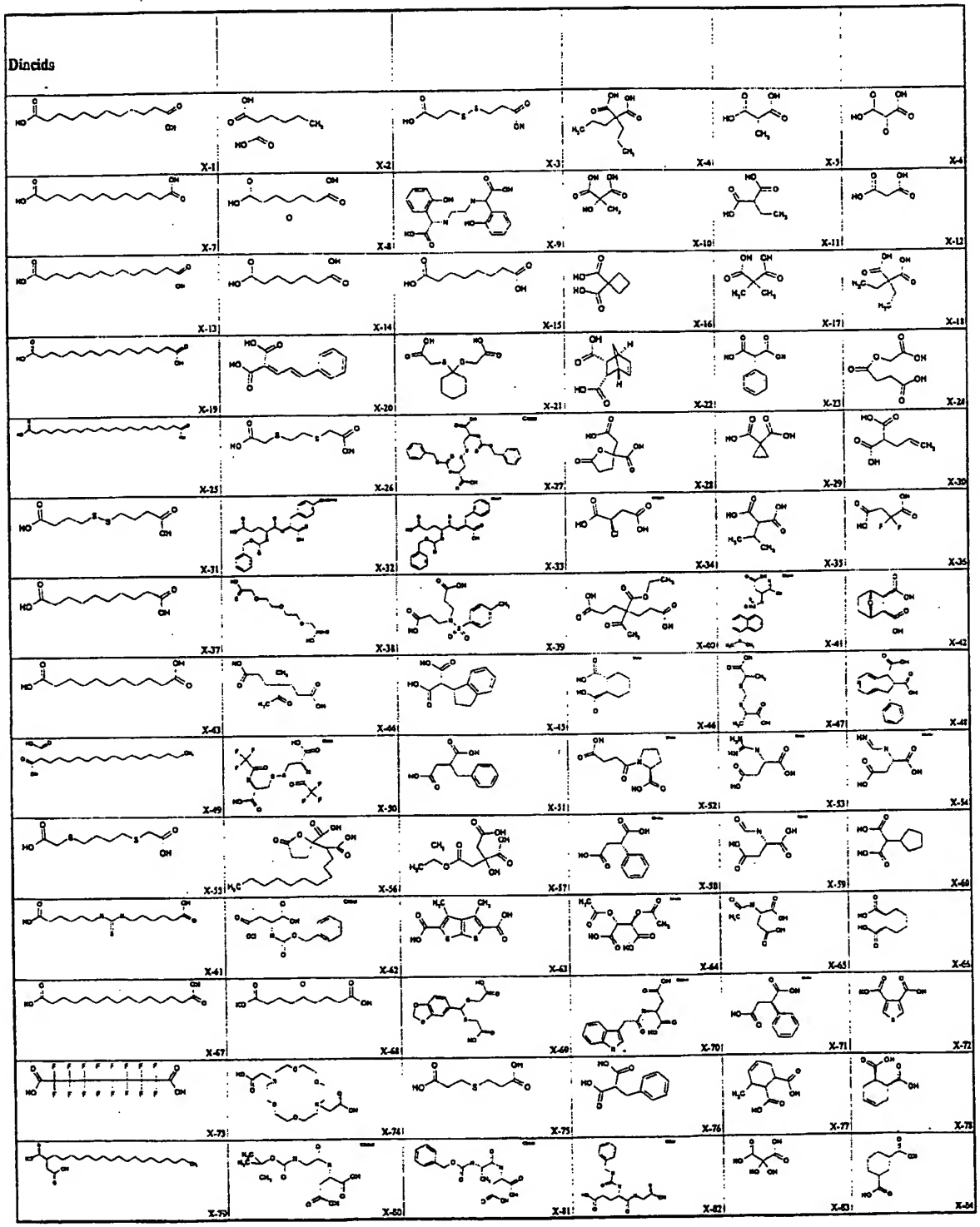
disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

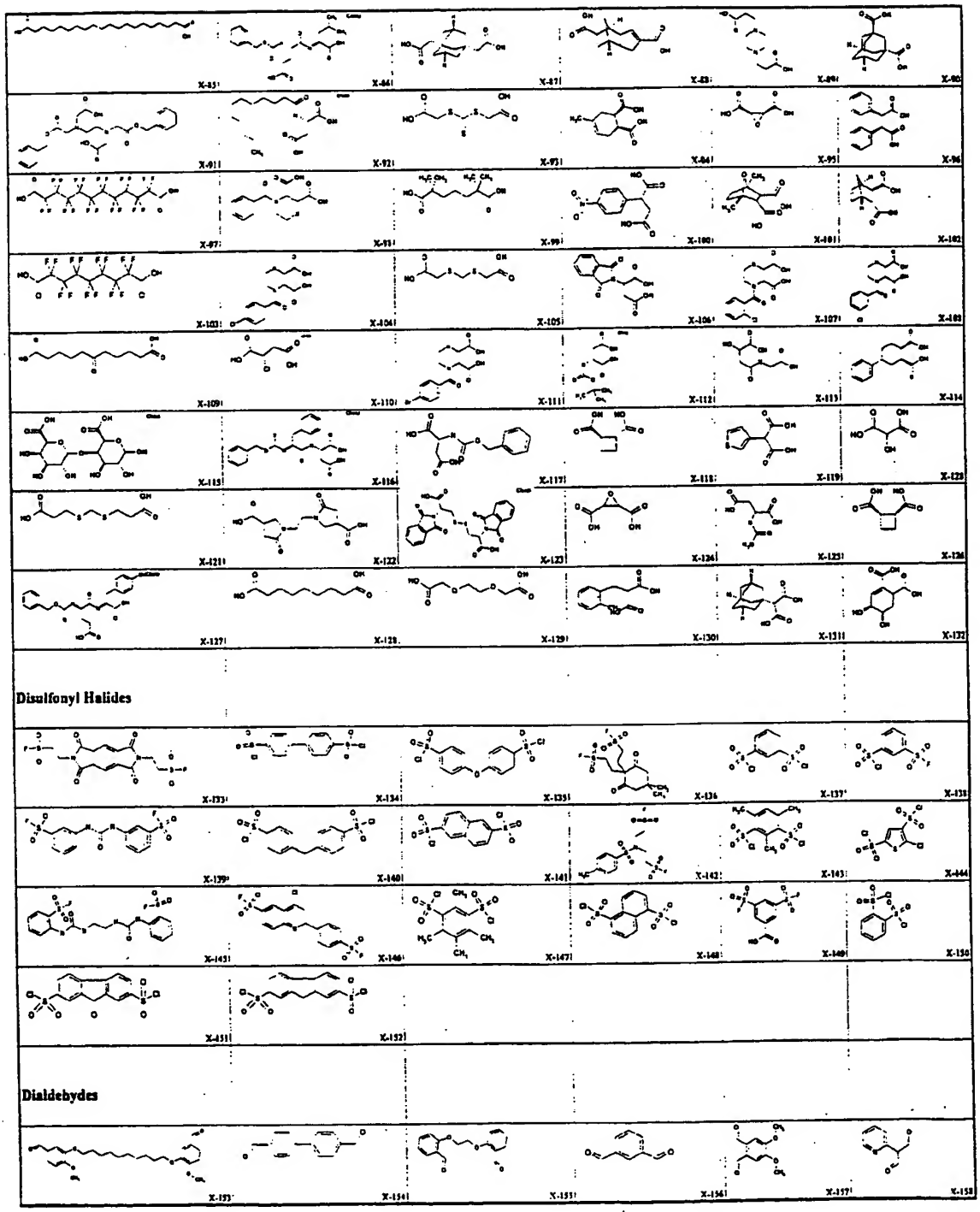
COMPLEMENTARY BINDING CHEMISTRIES

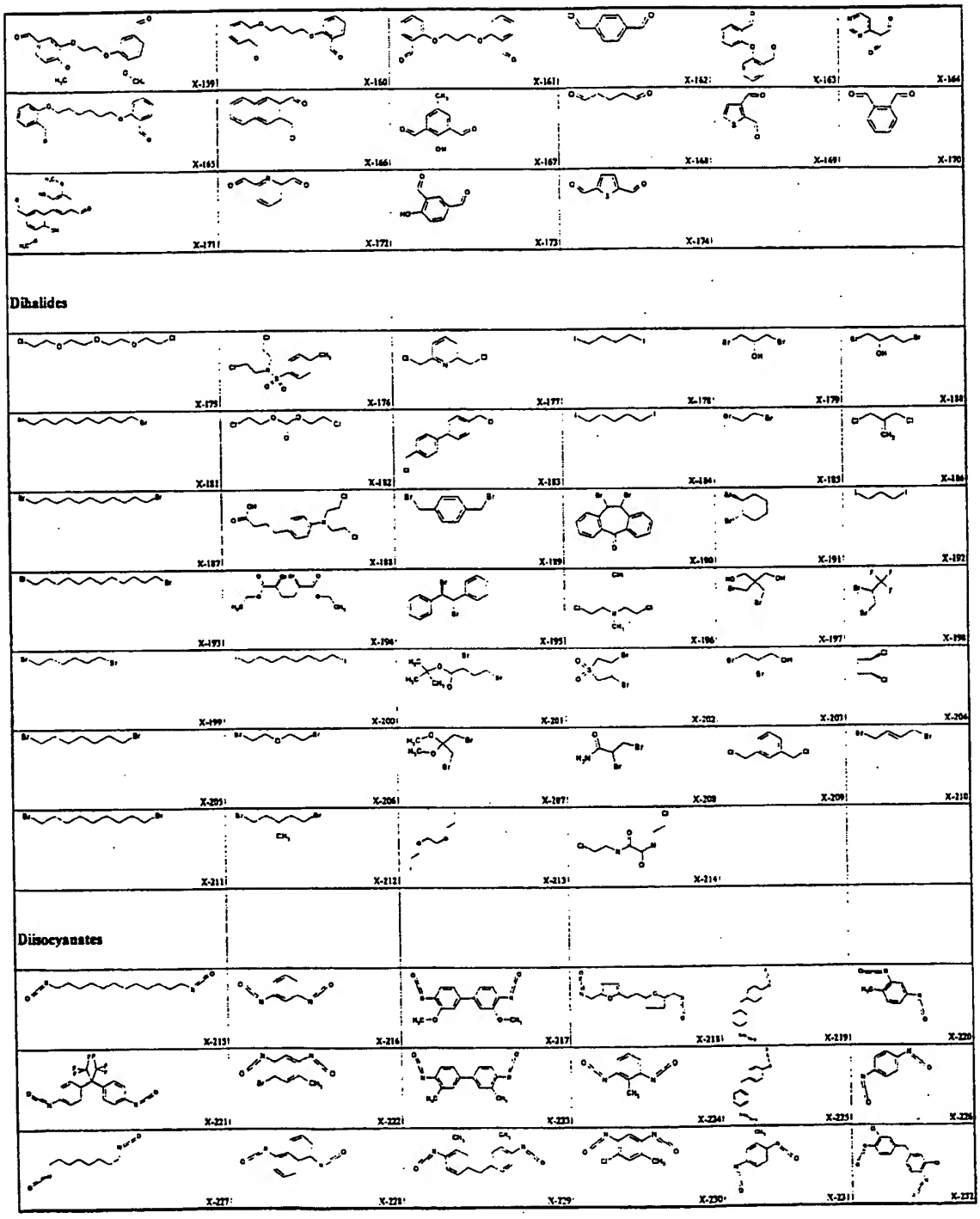
First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-aminohydroxy
sulfonyl halide	amine	sulfonamide
carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/$NaCNBH_3$	amine
ketone	amine/$NaCNBH_3$	amine
amine	isocyanate	urea

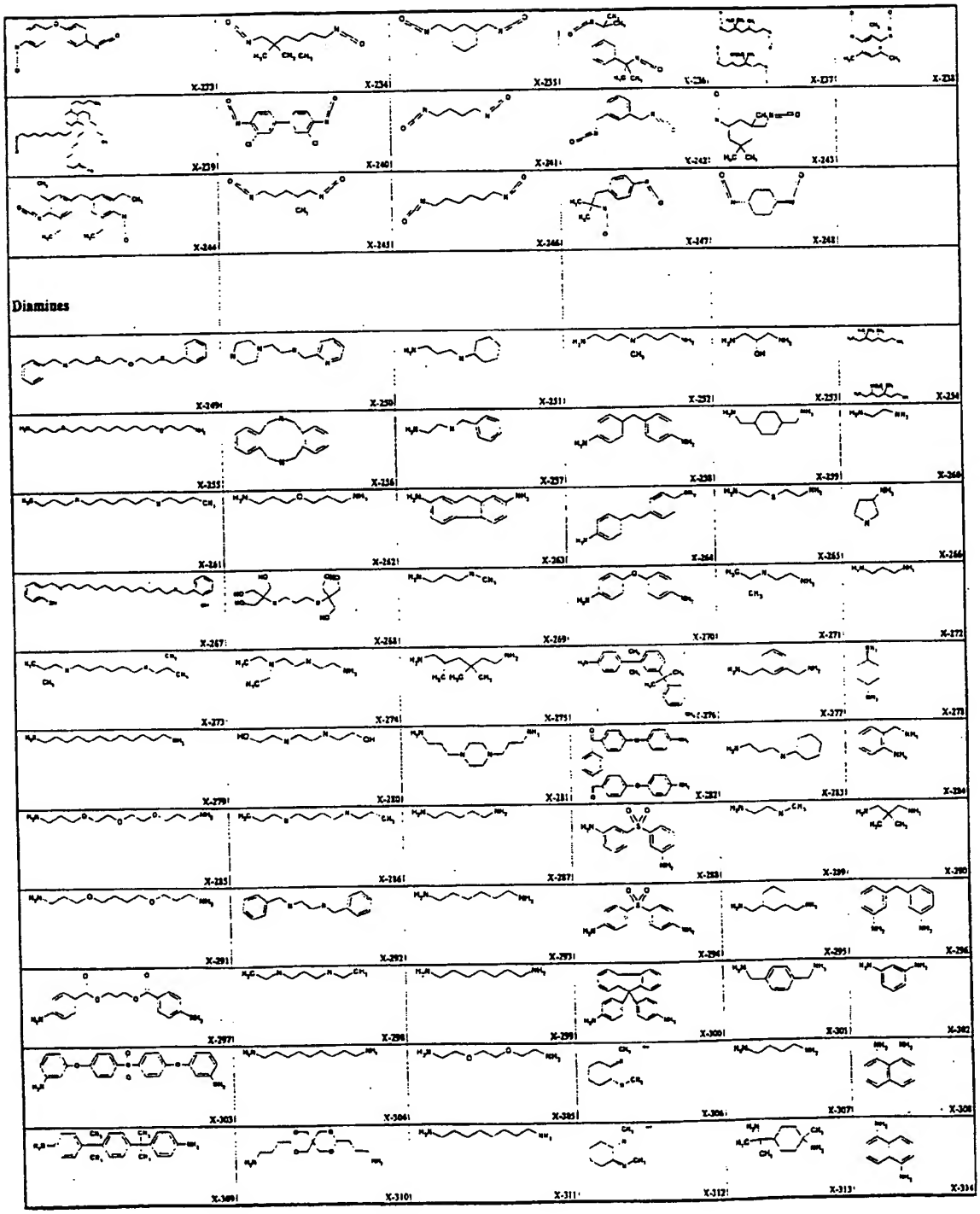
Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below.

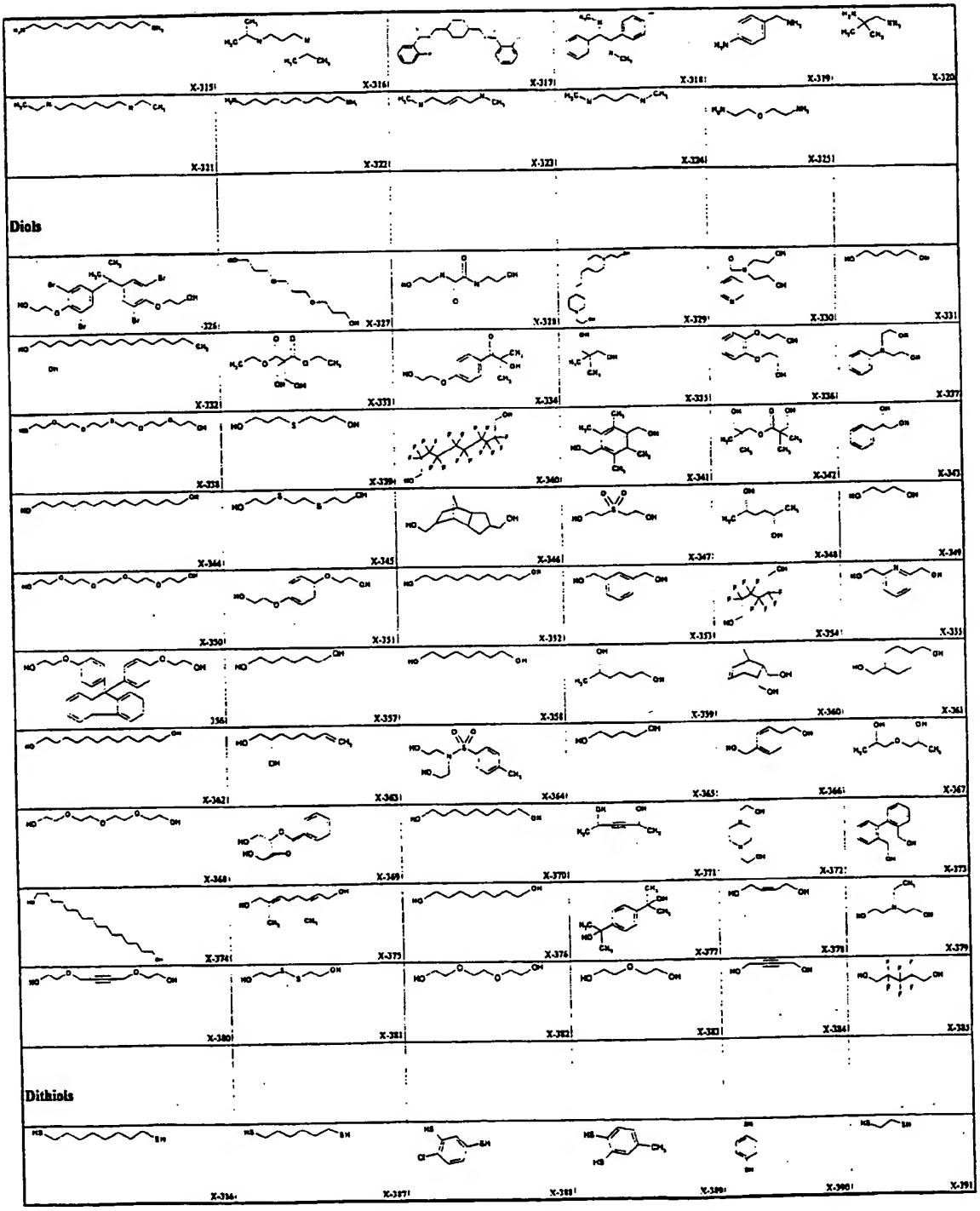
Dincids

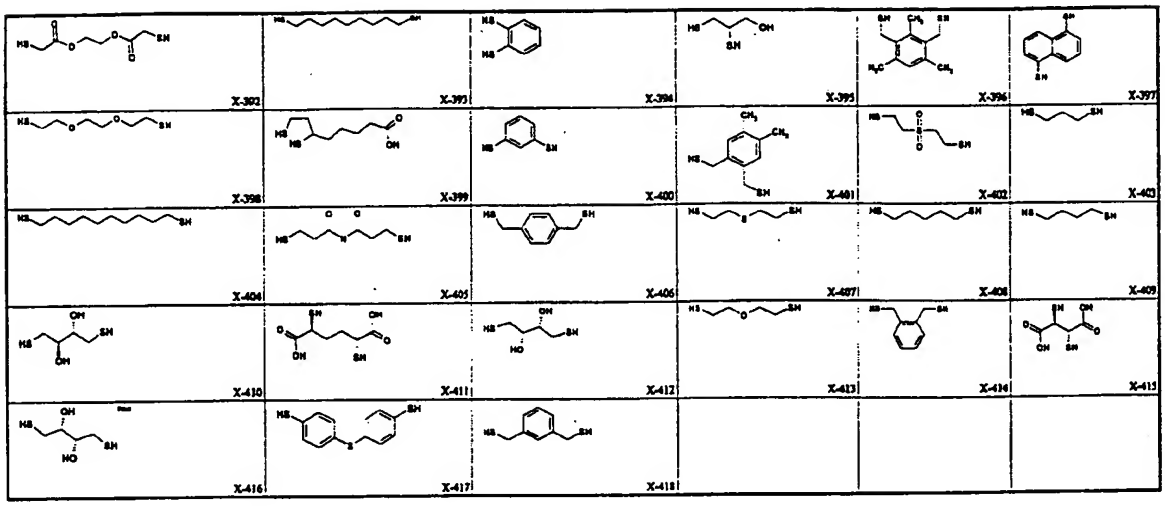



Disulfonyl Halides
Dialdehydes


Dihalides
Diisocyanates


Diamines


Diols
Dithiols


X-392
X-393
X-394
X-395
X-396
X-397
X-398
X-399
X-400
X-401
X-402
X-403
X-404
X-405
X-406
X-407
X-408
X-409
X-410
X-411
X-412
X-413
X-414
X-415
X-416
X-417
X-418

Representative ligands for use in this invention include, by way of example, L-1 through L-3 as identified above.

For example, L-1 can be a poison;
L-2 can be an intercalator; and
L3 can be a catalytic antogonist.

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 through L-3 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-

L-1/X-109- L-1/X-110- L-1/X-111- L-1/X-112- L-1/X-113- L-1/X-114-
L-1/X-115- L-1/X-116- L-1/X-117- L-1/X-118- L-1/X-119- L-1/X-120-
L-1/X-121- L-1/X-122- L-1/X-123- L-1/X-124- L-1/X-125- L-1/X-126-
L-1/X-127- L-1/X-128- L-1/X-129- L-1/X-130- L-1/X-131- L-1/X-132-
L-1/X-133- L-1/X-134- L-1/X-135- L-1/X-136- L-1/X-137- L-1/X-138-
L-1/X-139- L-1/X-140- L-1/X-141- L-1/X-142- L-1/X-143- L-1/X-144-
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L-1/X-223- L-1/X-224- L-1/X-225- L-1/X-226- L-1/X-227- L-1/X-228-
L-1/X-229- L-1/X-230- L-1/X-231- L-1/X-232- L-1/X-233- L-1/X-234-
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L-2/X-163 L-2/X-164 L-2/X-165 L-2/X-166 L-2/X-167 L-2/X-168
L-2/X-169 L-2/X-170 L-2/X-171 L-2/X-172 L-2/X-173- L-2/X-174-
L-2/X-175- L-2/X-176- L-2/X-177- L-2/X-178- L-2/X-179- L-2/X-180-
L-2/X-181- L-2/X-182- L-2/X-183- L-2/X-184- L-2/X-185- L-2/X-186-
L-2/X-187- L-2/X-188- L-2/X-189- L-2/X-190- L-2/X-191- L-2/X-192-
L-2/X-193- L-2/X-194- L-2/X-195- L-2/X-196- L-2/X-197- L-2/X-198-
L-2/X-199- L-2/X-200- L-2/X-201- L-2/X-202- L-2/X-203- L-2/X-204-
L-2/X-205- L-2/X-206- L-2/X-207- L-2/X-208- L-2/X-209- L-2/X-210-
L-2/X-211- L-2/X-212- L-2/X-213- L-2/X-214- L-2/X-215- L-2/X-216-

L-2/X-217- L-2/X-218- L-2/X-219- L-2/X-220- L-2/X-221- L-2/X-222-
L-2/X-223- L-2/X-224- L-2/X-225- L-2/X-226- L-2/X-227- L-2/X-228-
L-2/X-229- L-2/X-230- L-2/X-231- L-2/X-232- L-2/X-233- L-2/X-234-
L-2/X-235- L-2/X-236- L-2/X-237- L-2/X-238- L-2/X-239- L-2/X-240-
L-2/X-241- L-2/X-242- L-2/X-243- L-2/X-244- L-2/X-245- L-2/X-246-
L-2/X-247- L-2/X-248- L-2/X-249- L-2/X-250- L-2/X-251- L-2/X-252-
L-2/X-253- L-2/X-254- L-2/X-255- L-2/X-256- L-2/X-257- L-2/X-258-
L-2/X-259- L-2/X-260- L-2/X-261- L-2/X-262- L-2/X-263- L-2/X-264-
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L-2/X-277- L-2/X-278- L-2/X-279- L-2/X-280- L-2/X-281- L-2/X-282-
L-2/X-283- L-2/X-284- L-2/X-285- L-2/X-286- L-2/X-287- L-2/X-288-
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L-2/X-295- L-2/X-296- L-2/X-297- L-2/X-298- L-2/X-299- L-2/X-300-
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L-2/X-307- L-2/X-308- L-2/X-309- L-2/X-310- L-2/X-311- L-2/X-312-
L-2/X-313- L-2/X-314- L-2/X-315- L-2/X-316- L-2/X-317- L-2/X-318-
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L-2/X-337- L-2/X-338- L-2/X-339- L-2/X-340- L-2/X-341- L-2/X-342-
L-2/X-343- L-2/X-344- L-2/X-345- L-2/X-346- L-2/X-347- L-2/X-348-
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L-2/X-409- L-2/X-410- L-2/X-411- L-2/X-412- L-2/X-413- L-2/X-414-
L-2/X-415- L-2/X-416- L-2/X-417- L-2/X-418-

L-3/X-1- L-3/X-2- L-3/X-3- L-3/X-4- L-3/X-5- L-3/X-6-
L-3/X-7- L-3/X-8- L-3/X-9- L-3/X-10- L-3/X-11- L-3/X-12-
L-3/X-13- L-3/X-14- L-3/X-15- L-3/X-16- L-3/X-17- L-3/X-18-
L-3/X-19- L-3/X-20- L-3/X-21- L-3/X-22- L-3/X-23- L-3/X-24-
L-3/X-25- L-3/X-26- L-3/X-27- L-3/X-28- L-3/X-29- L-3/X-30-
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L-3/X-385- L-3/X-386- L-3/X-387- L-3/X-388- L-3/X-389- L-3/X-390-
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L-3/X-397- L-3/X-398- L-3/X-399- L-3/X-400- L-3/X-401- L-3/X-402-
L-3/X-403- L-3/X-404- L-3/X-405- L-3/X-406- L-3/X-407- L-3/X-408-
L-3/X-409- L-3/X-410- L-3/X-411- L-3/X-412- L-3/X-413- L-3/X-414-
L-3/X-415- L-3/X-416- L-3/X-417- L-3/X-418-

and so on.

Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of this invention are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds described herein associated with pharmaceutically acceptable carriers. In making the compositions

of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.001 to about 1 g, more usually about 1 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood, however, that the amount of the compound actually administered will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

As previously mentioned, normal dividing cells contain the same topoisomerases as that of neoplastic cells. Therefore, increasing the binding coefficient and the subsequent "duration of action" of the enzyme may have a significant negative impact on myelosuppression and gastrointestinal cell toxicity. To combat the issue of target selectivity, the enzyme antagonist could be delivered in an alternative fashion. That is, instead of a systemic administration of the drug, an intratumoral administration is employed. This method of delivery, however, would only be useful in the treatment of well-isolated solid tumors and would have no effect on liquid or disseminated neoplasms.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)

Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg
Total	120 mg

The active ingredient, starch and cellulose are passed through a No. 20 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders, which are then passed through a 16 mesh U.S. sieve. The granules so produced are dried at 50° to 60°C and passed through a 16 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 30 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as follows:

Ingredient	Amount
Active Ingredient	25 mg
Saturated fatty acid glycerides to	2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

A formulation may be prepared as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

Formulation Example 10

A topical formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. *See, e.g.*, U.S. Patent 5,023,252, issued June 11, 1991, herein incorporated by reference in its entirety. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences,* Mace Publishing Company, Philadelphia, PA, 17th ed. (1985).

Utility

The multibinding compounds of this invention inhibit topoisomerases which are essential enzymes in higher eucaryotes for DNA replication and repair. Accordingly, the multibinding compounds of this invention and pharmaceutical compositions comprising such compounds are useful in the treatment and prevention of cancer, [microbial infections] and the like.

When used in treating or ameliorating such conditions, the compounds of this invention are typically delivered to a patient in need of such treatment by a pharmaceutical composition comprising a pharmaceutically acceptable diluent and an effective amount of at least one compound of this invention. The amount of compound administered to the patient will vary depending upon what compound and/or composition is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions are administered to a patient already suffering from, for example, cancer, [microbial infections] in an amount sufficient to at least partially reduce cancer, [microbial infections] . Amounts effective for this use will depend on the judgment of the attending clinician depending upon factors such as the degree or severity of the cancer, [microbial infections] in the patient, the age, weight and general condition of the patient, and the like. The pharmaceutical compositions of this invention may contain more than one compound of the present invention.

As noted above, the compounds administered to a patient are in the form of pharmaceutical compositions described above which can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, etc.. These compounds are effective as both injectable and oral deliverable pharmaceutical compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

The multibinding compounds of this invention can also be administered in the form of pro-drugs, i.e., as derivatives which are converted into a biologically active compound *in vivo*. Such pro-drugs will typically include compounds in which, for example, a carboxylic acid group, a hydroxyl group or a thiol group is converted to a biologically labile group, such as an ester, lactone or thioester group which will hydrolyze *in vivo* to reinstate the respective group.

The following synthetic and biological examples are offered to illustrate this invention and are not to be construed in any way as limiting the scope of this invention. As will be readily apparent to those of ordinary skill in the art, the synthetic procedures described herein or those known in the art may be readily modified to afford a wide variety of compounds within the scope of this invention. Unless otherwise stated, all temperatures are in degrees Celsius.

EXAMPLES

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

Å	=	Angstroms
cm	=	centimeter
DCC	=	dicyclohexyl carbodiimide
DMF	=	*N,N*-dimethylformamide
DMSO	=	dimethylsulfoxide
EDTA	=	ethylenediaminetetraacetic acid
g	=	gram
HBSS	=	Hank's Balanced Salt Solution
HPLC	=	high performance liquid chromatography
MEM	=	minimal essential medium
mg	=	milligram
MIC	=	minimum inhibitory concentration
min	=	minute
mL	=	milliliter
mm	=	millimeter
mmol	=	millimol
N	=	normal

NADP	=	nicotinamide adenine dinucleotide phosphate
NADPH	=	nicotinamide adenine dinucleotide phosphate, reduced form
THF	=	tetrahydrofuran
μL	=	microliters
μm	=	microns

Example 1 illustrates the synthesis of an intermediate that is useful in preparing compounds of this invention.

Example 1

Preparation of Synthon D as illustrated in Fig. 12.

To a solution of 68.1 mmol of sodium ethoxide in 150 mL of absolute ethanol is added 68.02 mmol of L-5-hydroxytryptophanol in 100 mL of ethanol and 74.8 mmols of diethyl carbonate. The solution is refluxed for 5 hr. then concentrated. The residue is partitioned between sat. NH_4Cl and methylene chloride and the aqueous phase again extracted with methylene chloride. The combined organic phases are dried over sodium sulfate and the solvent removed. The product is purified by chromatography and/or crystallization. To a solution of 2 mmols of the above oxazolidinone intermediate in 8 mL of anhydrous THF with 3 mmols of syringaldehyde dimethyl acetal is added 0.2 mmols of anhydrous trifluoroacetic acid and the solution heated to reflux. The reaction is followed by TLC and when judged complete partitioned between sat. sodium bicarbonate and methylene chloride. The organic phase is dried over sodium sulfate and the solvent removed. The residue is purified by crystallization or chromatography to afford Synthon D. Figure 13 shows the structures of azatoxin and four derivatives thereof, namely, Synthons D, E, F, and G. The synthesis of azatoxin is described in Madalengoitia, et al., Bioorg. Med. Chem., 1997 5, 1807.

Employing the synthesis illustrated in Figure 12 and described above, Synthons E,F, and G as shown in Figure 13 can also be prepared, but the starting

material used would be one of the compounds shown in Figure 14. Specifically, for synthesizing Synthon E, the compound is one in which R_7 is OC_6H_5 and R_4, R_5 and R_6 is H. Similarly, for Synthons F and G, the compound is one in which R_4 is NH_2 and R_6 is NH_2, respectively, with the other R groups being H.

Examples 2-13 illustrate the preparation of compounds of formula I.

Example 2

Diamine linked compounds as illustrated in Figure 15.

A solution of 1.2 mmols of Synthon A in 15 mL of anhydrous THF is treated with 2.4 mmols of anhydrous barium carbonate and 0.6 mmols of 1,4-phenylenediamine and kept overnight at room temperature. After filtering and concentrating, the residue is purified by chromatography to afford Structure 1. Synthon A is described in Lee et al., J. Med. Chem., 1996, 29, 1547. See also, Lee, et al., *J. Med. Chem.*, 1990, 33 1364, which describes the reaction of Synthon A with aniline to form monomeric compounds.

Example 3

Diol linked compounds as illustrated in Figure 16.

A solution of 1.2 mmols of Synthon A in 15 mL of anhydrous THF is treated with 2.4 mmols of anhydrous barium carbonate and 0.6 mmols of tetraethylene glycol and kept overnight at room temperature. After filtering and concentrating, the residue is purified by chromatography to afford Structure 2.

Example 4

Dicarboxylic acid linked compounds as illustrated in Figure 17.

To a solution of 100 mmols of ethylene diamine in 200 mL of anhydrous THF is slowly added 10 mmols of suberyl chloride with good agitation. After 1hr., the mixture is concentrated and the residue is partitioned between ethyl acetate and sat. sodium bicarbonate. The aqueous phase is extracted with ethyl

acetate and the combined organic layers washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford intermediate 1. A solution of 1.2 mmols of Synthon A in 15 mL of anhydrous THF is treated with 2.4 mmols of anhydrous barium carbonate and 0.6 mmols of intermediate 1 and kept overnight at room temperature. After filtering and concentrating, the residue is purified by chromatography to afford Structure 3.

Example 5

Dialdehyde linked compounds as illustrated in Figure 18.

A solution of 20 mmols of Synthon B in 50 mL of toluene with 10 mmols of isophthalaldehyde and 100 mg of p-TSA is refluxed with the azeotropic removal of water. The reaction is followed by TLC and when judged complete, is cooled and washed with sat. sodium bicarbonate, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 4. Synthon B is described in Long et al., *Cancer Chemother Pharmacol.*, 1994, 34 (suppl.), S26.

Example 6

Dihalo linked compounds as illustrated in Figure 19.

A solution of 50 mmols of Synthon C in 20 mL of DMF with 25 mmols of 1,6-dibromohexane and 50 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 5. Synthon C. is described in Lee, et al., *J. Nat. Prod.*, 1992, 55, 1100.

Example 7

Bis mesylate linked compounds as illustrated in Figure 20.

A solution of 50 mmols of α - Peltatin in 20 mL of DMF with 25 mmols of the bis mesylate of hexaethylene glycol (prepared in the usual way from hexaethylene glycol and methanesulfonyl chloride) and 50 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 6.

Example 8

Dicarboxylic acid linked compounds as illustrated in Figure 21.

To a solution 20 mmols of Synthon F in 50 mL of ethyl acetate with 20 mmols of triethylamine is added 10 mmols of diglycolyl chloride. After 1hr., the reaction is washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 7.

Example 9

Dihalo linked compounds as illustrated in Figure 22.

A solution of 20 mmols of Synthon D in 20 mL of DMF with 10 mmols of αα'-dibromo-p-xylene and 20 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 8.

Example 10

Halo carboxylic acid linked compounds as illustrated in Figure 23.

A solution of 20 mmols of Synthon F in 50 mL of ethyl acetate with 20 mmols of triethylamine is treated with 20 mmols of 6-bromohexanoly chloride at room temperature. After 1hr., the reaction is washed with water, dried over

sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford intermediate 2. A solution of 10 mmols of Synthon D in 10 mL of DMF with 10 mmols of intermediate 2 and 10 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 9.

Example 11

Diol linked compounds as illustrated in Figure 24.

A mixture of 15 mmols of Synthon H, 10 mmols of hexaethylene glycol and 4 mL of N,N-dimethylformamide dineopentyl acetal is kept at 115-120 C for 24hr. After cooling, the reaction is diluted with ethyl acetate, washed with water, dried over sodium sulfate and the solvent removed. The residue is purified by chromatography to afford Structure 10 and the monoalkylated derivative, intermediate 3, which will be used in example 13. See Guthrie, et al., *J. Med. Chem.*, 1975, 18, 755, which describes the Synthesis of monomeric derivatives of Synthon H.

The following Examples 12 and 13 describe the syntheses of heterodimers comprising two nonidentical ligands.

Example 12

Diol linked compounds as illustrated in Figure 25.

To a well stirred mixture of 40 mmols of anhydrous barium carbonate in 100mL anhydrous THF with 100 mmols of triethlyene glycol is slowly added 20 mmols of Synthon A. After 24 hr., the mixture is concentrated and purified by chromatography to afford intermediate 4. A mixture of 15 mmols of Synthon H, 20 mmols intermediate 4 and 4 mL of N,N-dimethylformamide dineopentyl

acetal is kept at 115-120 C for 24hr. After cooling, the reaction is diluted with ethyl acetate, washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 11.

Example 13

Diol linked compounds as illustrated in Figure 26.

Intermediate 3 is converted to the corresponding mesylate in the usual was with methanesulfonyl chloride and N-methylmorpholine in methylene chloride and is purified by chromatography as necessary. A mixture of 20 mmols each of the above mesylate, Synthon C and potassium carbonate in 25 mL of DMF is warmed as necessary and the reaction followed by TLC When judged complete, the reaction is partitioned between isopropyl acetate and water and the organic layer repeatedly washed with water then dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford Structure 12.

Testing

The bivalent agents of the invention are useful in medical treatments and exhibit biological effects that can be demonstrated in vitro in assays well known to those skilled in the art. For example, the cytoxic effect of bivalent compounds is evaluated in cultures of murine leukemia cells (Daley, L., et al., J. Med. Chem. 1998. 41(23):4475). The in vitro effects of agents on topoisomerase II activity are also determined in a murine leukemia model (Daley, L., et al., J. Med. Chem. 1998. 41(23):4475).

The bivalent ligands of the invention are assessed in vivo in assays well known to those skilled in the art. For example, the effect of the compounds on tumors in mice is determined following the method of Daley et al. (Daley, L., et al., J. Med. Chem. 1998. 41(23):4475). The anti-tumor effect of the

compounds is also evaluated in human soft-tissue sarcoma, breast carcinoma, lung tumor and other neoplastic tissue xenografts (Boven, E., et al. Br. J. Cancer. 1998. 78(12):1686, Kraus-Berthier, L., et al. Eur. J. Cancer 33(11):1881, and Taetle, R., et al., Cancer Treatment Reports. 71(3):297). In vivo antitumor activity is also studied in a murine leukemia model (Daley, L., et al., J. Med. Chem. 1998. 41(23):4475).

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

All of the publications, patent applications and patents cited in this application are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

WHAT IS CLAIMED IS:

1. A multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is about 2-50Å; and pharmaceutically acceptable salts thereof.

2. The multibinding compound of claim 1 wherein said ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

3. The multibinding compound of claim 1 which has 2 ligands.

4. A multibinding compound of formula I:

$(L)_p(X)_q$ I

wherein each L is independently a ligand capable of binding to a topoisomerase; each X is independently a linker; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; and further wherein the distance between ligands is at least 10Å; and pharmaceutically acceptable salts thereof.

5. The multibinding compound of Claim 4 wherein *q* is less than *p*.

6. The multibinding compound of Claim 5 wherein each ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

7. The multibinding compound of Claim 6 wherein each linker independently has the formula:

$$-X^a-Z-(Y^a-Z)_m-Y^b-Z-X^a-$$

wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, $-S(O)_n$CR'R"-, $-S(O)_n$-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

8. The multibinding compound of claim 4 wherein p is 2 and q is 1.

9. A multibinding compound of formula II:

$$L'-X'-L' \qquad II$$

wherein each L' is independently a ligand capable of binding to a topoisomerase; and X' is a linker and further wherein the distance between ligands is between about 2-50Å; and pharmaceutically- acceptable salts thereof.

10. The multibinding compound of Claim 9 wherein each ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

11. The multibinding compound of Claim 10 wherein X' has the formula:

$$-X^a-Z-(Y^a-Z)_m-Y^b-Z-X^a-$$

wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -$S(O)_n$CR'R"-, -$S(O)_n$-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is between about 2-50Å; and pharmaceutically acceptable salts thereof.

13. The pharmaceutical composition of claim 12 wherein said ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-

10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

14. The pharmaceutical composition of claim 12 wherein the multibinding compound has two ligands.

15. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound of formula I:

$$(L)_p(X)_q \qquad I$$

wherein each L is independently a ligand capable of binding to a topoisomerase; each X is independently a linker; *p* is an integer of from 2 to 10; and *q* is an integer of from 1 to 20; and further wherein the distance between ligands is between about 2-50Å; and pharmaceutically acceptable salts thereof.

16. The pharmaceutical composition of Claim 15 wherein said ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates),

elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

17. The pharmaceutical composition of Claim 15 wherein *q* is less than *p*.

18. The pharmaceutical composition of Claim 15 wherein *p* is 2 and *q* is 1.

19. The pharmaceutical composition of Claim 15 wherein each linker independently has the formula:

$$-X^a\text{-}Z\text{-}(Y^a\text{-}Z)_m\text{-}Y^b\text{-}Z\text{-}X^a\text{-}$$

wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene,

alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-, -$S(O)_n$CR'R"-, -$S(O)_n$-NR'-, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

20. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and an effective amount of a multibinding compound of formula II:

$$L'-X'-L' \qquad II$$

wherein each L' is independently a ligand capable of binding to a topoisomerase; and X' is a linker and further wherein the distance between ligands is between about 2-50Å; and pharmaceutically acceptable salts thereof.

21. The pharmaceutical composition of Claim 20 wherein each ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-

1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

22. The pharmaceutical composition of Claim 21 wherein X' has the formula:

$$-X^a\text{-}Z\text{-}(Y^a\text{-}Z)_m\text{-}Y^b\text{-}Z\text{-}X^a\text{-}$$

wherein

m is an integer of from 0 to 20;

X^a at each separate occurrence is selected from the group consisting of -O-, -S-, -NR-, -C(O)-, -C(O)O-, -C(O)NR-, -C(S), -C(S)O-, -C(S)NR- or a covalent bond where R is as defined below;

Z is at each separate occurrence is selected from the group consisting of alkylene, substituted alkylene, cycloalkylene, substituted cylcoalkylene, alkenylene, substituted alkenylene, alkynylene, substituted alkynylene, cycloalkenylene, substituted cycloalkenylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y^a and Y^b at each separate occurrence are selected from the group consisting of -C(O)NR'-, -NR'C(O)-, -NR'C(O)NR'-, -C(=NR')-NR'-, -NR'-C(=NR')-, -NR'-C(O)-O-, -N=C(X^a)-NR'-, -P(O)(OR')-O-,

$-S(O)_nCR'R''-$, $-S(O)_n-NR'-$, -S-S- and a covalent bond; where *n* is 0, 1 or 2; and R, R′ and R″ at each separate occurrence are selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

23. A method for treating cancer or microbial infections in a mammal comprising administering to said mammal an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a multibinding compound comprising from 2 to 10 ligands covalently attached to one or more linkers, wherein each of said ligands independently is capable of binding to a topoisomerase and further wherein the distance between ligands is between about 2-50Å; and pharmaceutically acceptable salts thereof.

24. The method of claim 23 wherein said ligand is independently selected from the group consisting of A-62176, A-74932, acidine carboxamides, actinomycin D, AD-312, AD-347, AHMA, AMP-53, amrubicin, amsacrine, anthracyclines, asulacrine, azonafide, azatoxin, BBR-2778, BMY-43748, BO-2367, bromodeoxyuridine, C-1310, C-1311, CC-131, CJ-12373, CI-937, CI-920 (fostriecin), CP-115953, camptothecin, daunorubicin, doxorubicin, DuP 937 (losoxathrone), DuP 941, elinafide, elipticine-estradiol (conjugates), elsamitrucin, ER-37328, etoposide, fleroxacin, GI-149893, GL-331, GR-1222222X, ICRF-154, ICRF-193, idarubicin, iododoxorubicin, IST-622, KRQ-10018, intoplicine, lomofloxacin, losoxantrone, m-AMSA, merbarone, merabone, mitonafide, mitoxantrone, morindone, NCA-0465, NK-109, NK-611, NSC-655649, NSC-665517, NSC-675967, pazelliptine, pazufloxacin, PD-131112, piroxantrone, pyridobenzophenoxazine, S-16020-2, saintopin, sitafloxacin hydrate, SN-22995, sobuzoxane, SR-103, TAS-103, teloxantrone, teniposide, TLC-D-99, top-53, topotecan, tosufloxacin, TRK-710, trovafloxacin, UCE-6, VM-26, VP-16, W5R, WIN-33377, WIN-58161, WIN-645593, WQ-

2743, WQ-3034, WR-63320, XR-5942, XR-5000, and 773U82, and their analogues.

25. The method of claim 23 wherein the multibinding compounds has 2 ligands.

26. A method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties for topoisomerase.

27. A method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d) assaying the multimeric ligand compounds produced in the library prepared in (c) above to identify multimeric ligand compounds possessing multibinding properties for topoisomerase.

28. The method according to Claim 26 or 27, wherein the preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b).

29. The method according to Claim 28, wherein the multimeric ligand compounds comprising the multimeric ligand compound library are dimeric.

30. The method according to Claim 29, wherein the dimeric ligand compounds comprising the dimeric ligand compound library are heterodimeric.

31. The method according to Claim 30, wherein the heterodimeric ligand compound library is prepared by sequential addition of a first and second ligand.

32. The method according to Claim 26 or 27, wherein, prior to procedure (d), each member of the multimeric ligand compound library is isolated from the library.

33. The method according to Claim 32, wherein each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

34. The method according to Claim 26 or Claim 27, wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

35. The method according to Claim 34, wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

36. The method according to Claim 35, wherein the linkers are selected to have different linker lengths ranging from about 2-50Å.

37. The method according to Claim 26 or 27, wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

38. The method according to Claim 37, wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

39. The method according to Claim 26 or Claim 27, wherein the multimeric ligand compound library comprises homomeric ligand compounds.

40. The method according to Claim 26 or Claim 27, wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

41. A library of multimeric ligand compounds which may possess multivalent properties for topoisomerase, which library is prepared by the method comprising:

(a) identifying a ligand or a mixture of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

42. A library of multimeric ligand compounds which may possess multivalent properties for topoisomerase, which library is prepared by the method comprising:

(a) identifying a library of ligands wherein each ligand contains at least one reactive functionality;

(b) identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c) preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

43. The library according to Claim 41 or Claim 42, wherein the linker or linkers employed are selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers.

44. The library according to Claim 43, wherein the linkers comprise linkers of different chain length and/or having different complementary reactive groups.

45. The library according to Claim 44, wherein the linkers are selected to have different linker lengths ranging from about 2-50Å.

46. The library according to Claim 41 or 42, wherein the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands.

47. The library according to Claim 46, wherein said reactive functionality is selected from the group consisting of carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates, and precursors thereof wherein the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

48. The library according to Claim 41 or Claim 42, wherein the multimeric ligand compound library comprises homomeric ligand compounds.

49. The library according to Claim 41 or Claim 42, wherein the multimeric ligand compound library comprises heteromeric ligand compounds.

50. An iterative method for identifying multimeric ligand compounds possessing multibinding properties for topoisomerase, which method comprises:

(a) preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b) assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties for topoisomerase;

(c) repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties for topoisomerase;

(d) evaluating what molecular constraints imparted or are consistent with imparting multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e) creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

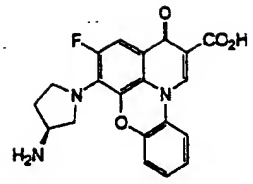
(f) evaluating what molecular constraints imparted or are consistent with imparting enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g) optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

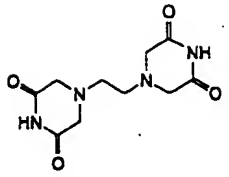
51. The method according to Claim 50, wherein steps (e) and (f) are repeated from 2-50 times.

52. The method according to Claim 51, wherein steps (e) and (f) are repeated from 5-50 times.

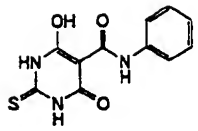
FIG. 1



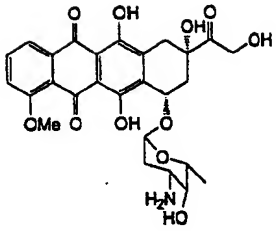
A-62176



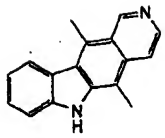
ICRF-154



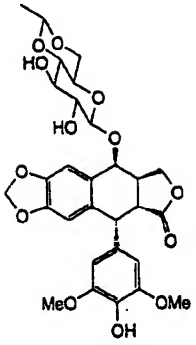
Merbarone



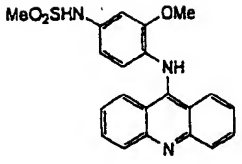
Doxorubicin



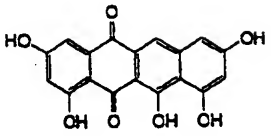
Ellipticine



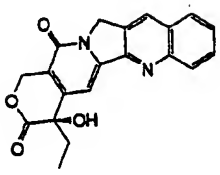
Etoposide



Amsacrine



Saintopin



Camptothecin

FIGURE 2

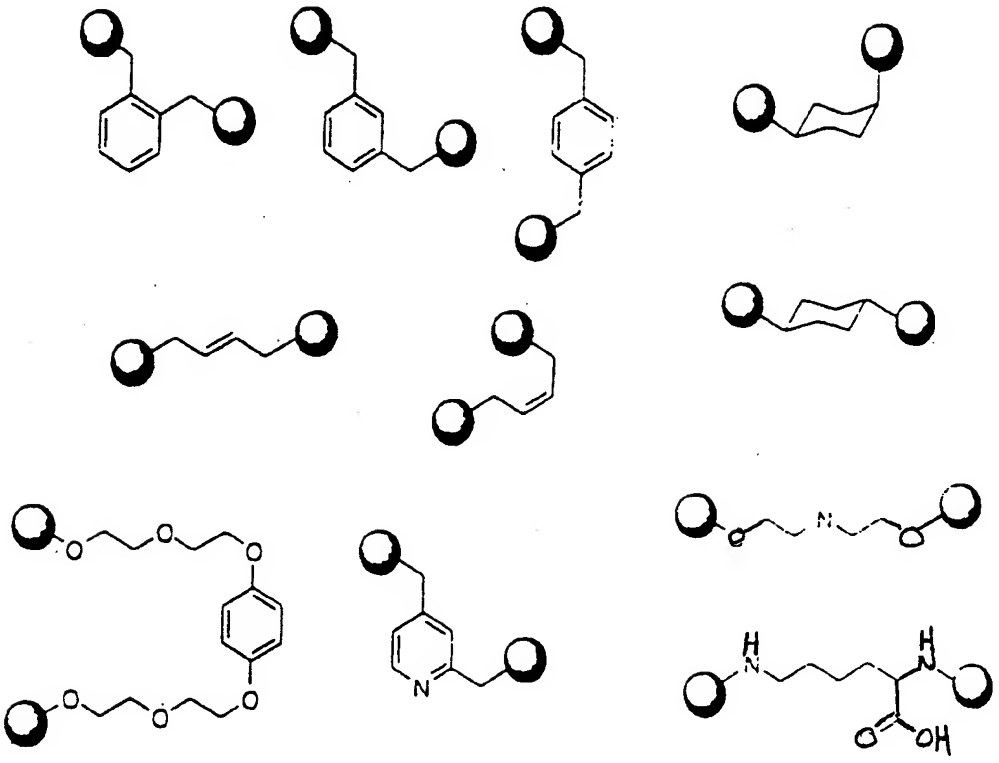


FIGURE 3

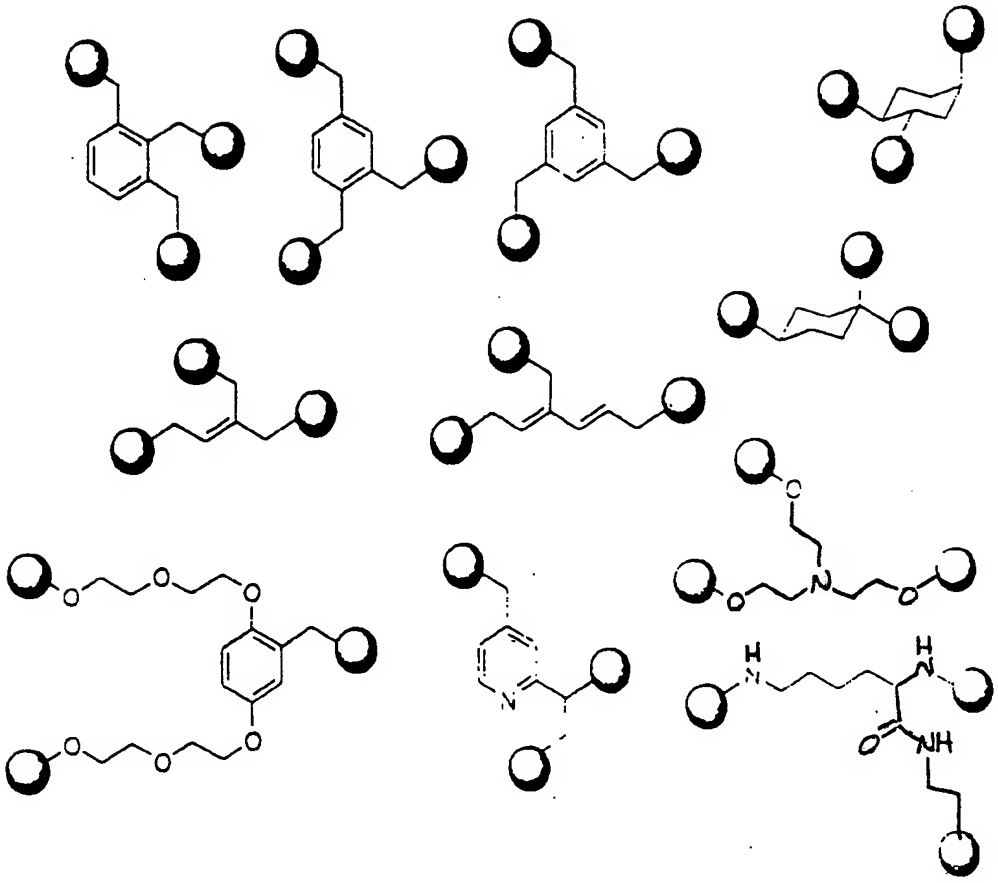


FIGURE 4

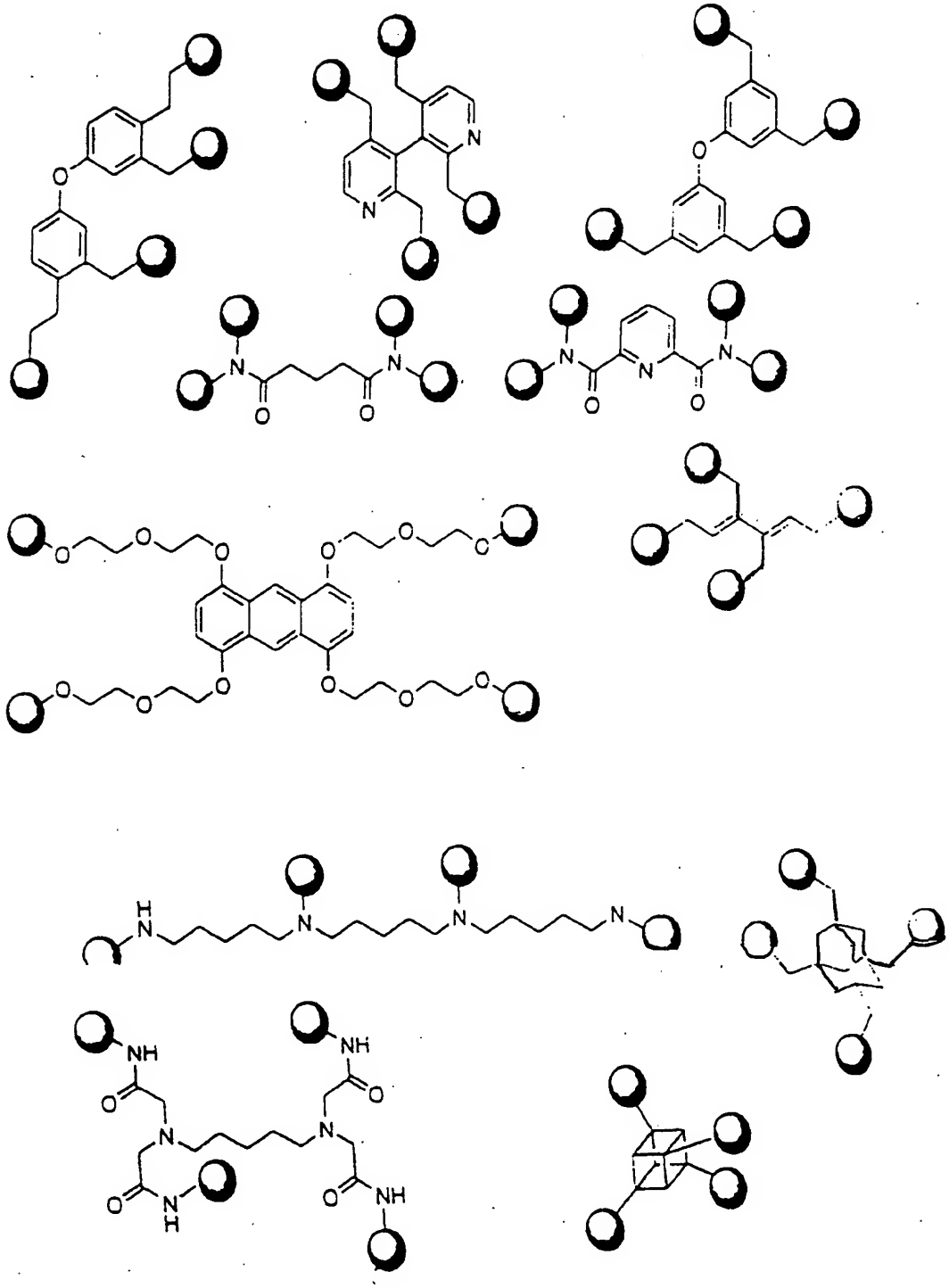
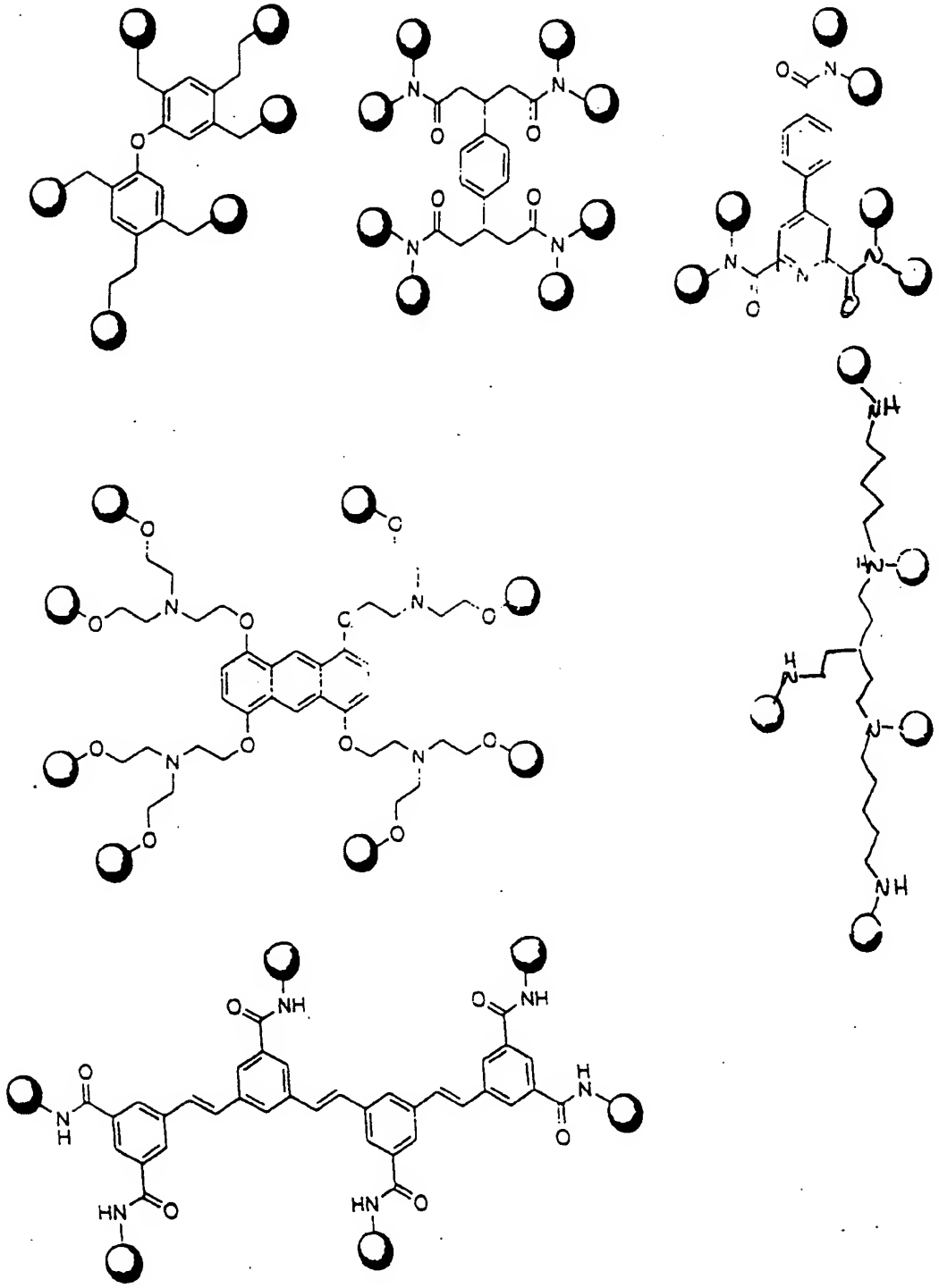


FIGURE 5



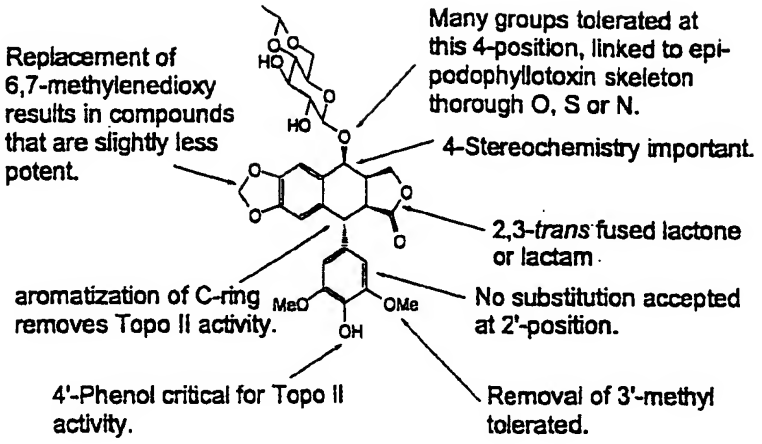
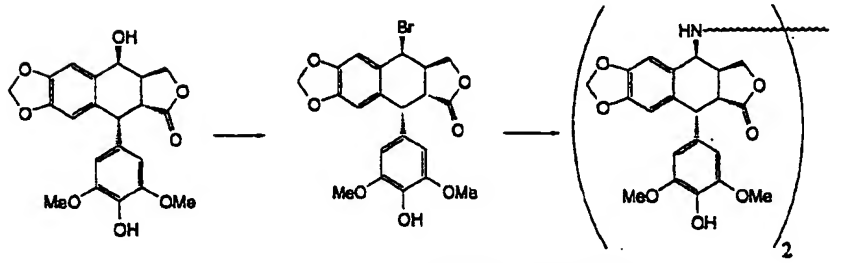
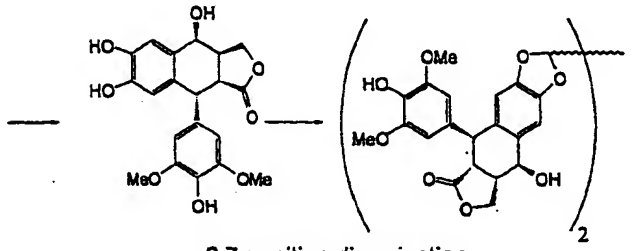

Replacement of 6,7-methylenedioxy results in compounds that are slightly less potent.
Many groups tolerated at this 4-position, linked to epi-podophyllotoxin skeleton thorough O, S or N.
4-Stereochemistry important.
2,3-*trans* fused lactone or lactam
No substitution accepted at 2'-position.
Removal of 3'-methyl tolerated.
4'-Phenol critical for Topo II activity.
aromatization of C-ring removes Topo II activity.

FIG 6


4-position dimerisation


6,7-position dimerisation

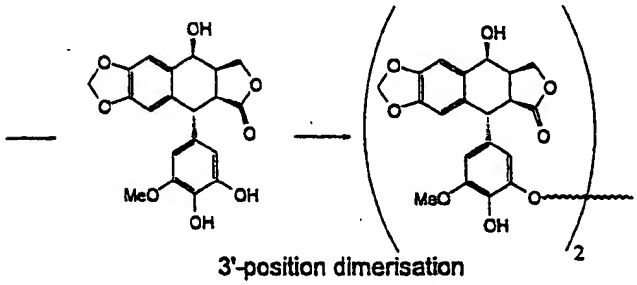

3'-position dimerisation

FIG 7

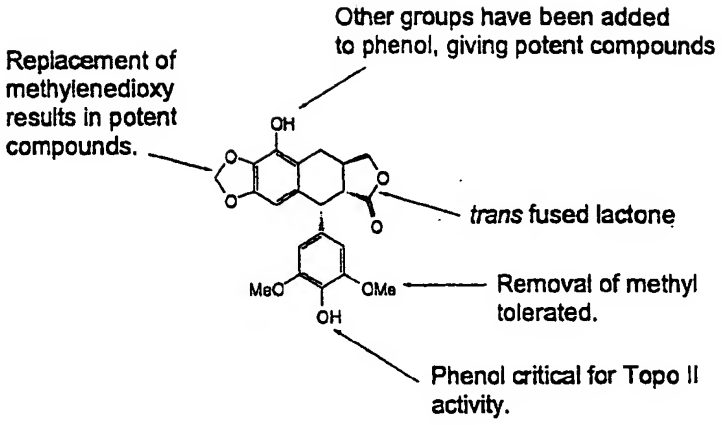

Other groups have been added to phenol, giving potent compounds
Replacement of methylenedioxy results in potent compounds.
OH
trans fused lactone
MeO
OMe
OH
Removal of methyl tolerated.
Phenol critical for Topo II activity.

FIG. 8

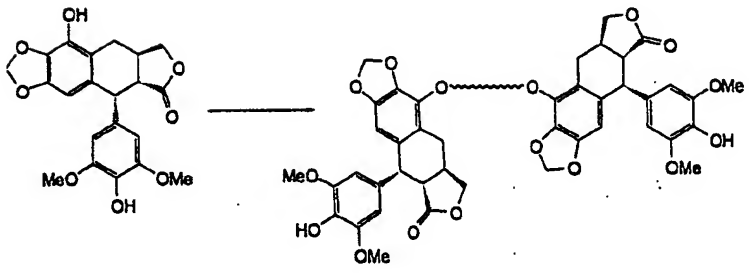

OH
MeO
OMe
OH
MeO
HO
OMe
OMe
OH
OMe

FIG. 9

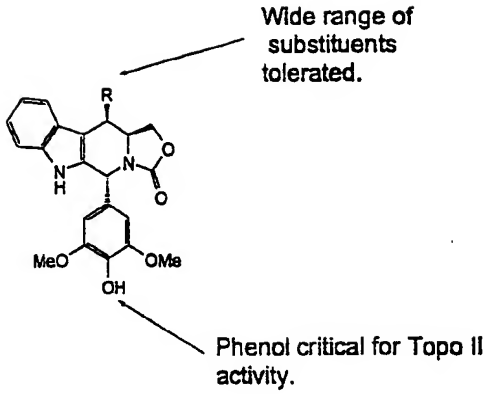

Wide range of substituents tolerated.
R
N
H
N
O
O
MeO
OMe
OH
Phenol critical for Topo II activity.

FIG. 10

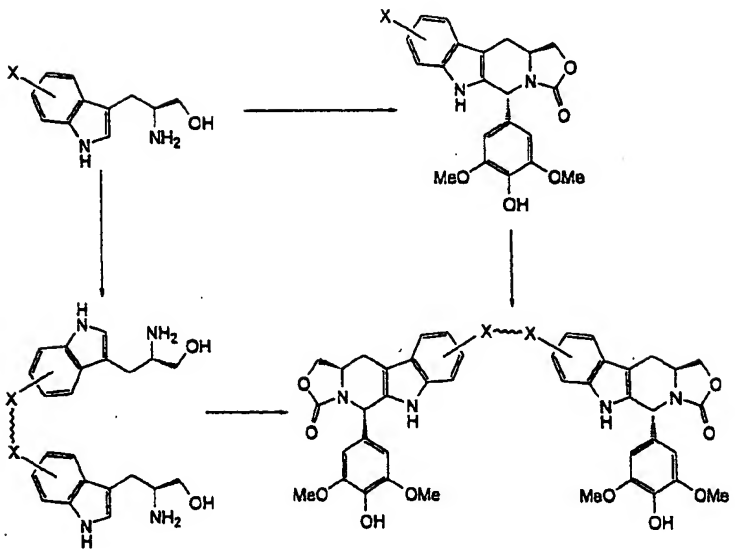

X
N
H
NH2
OH
O
MeO
OMe

FIG. 11

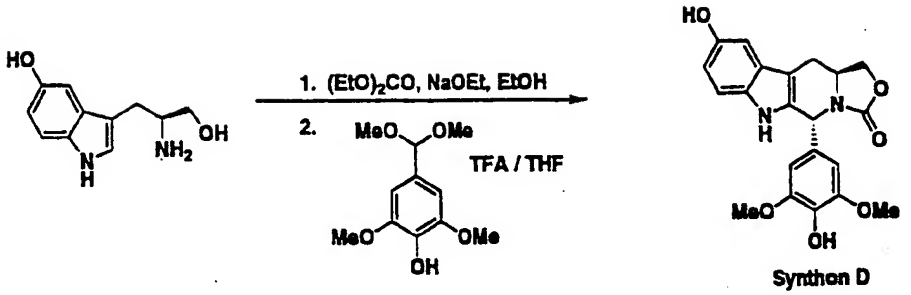


FIG. 12

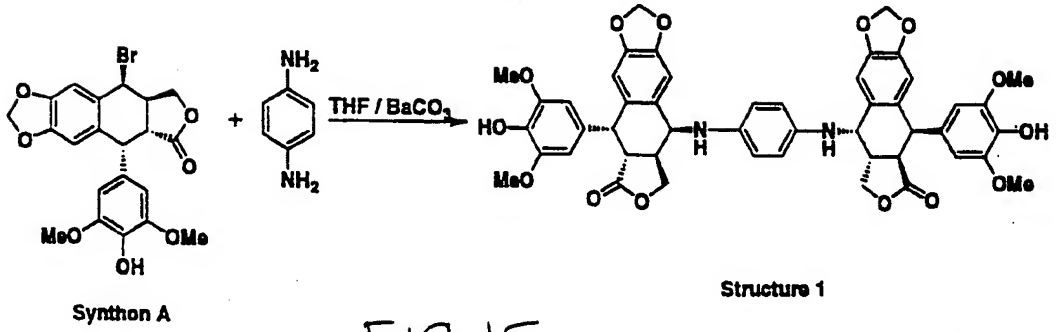


FIG. 15

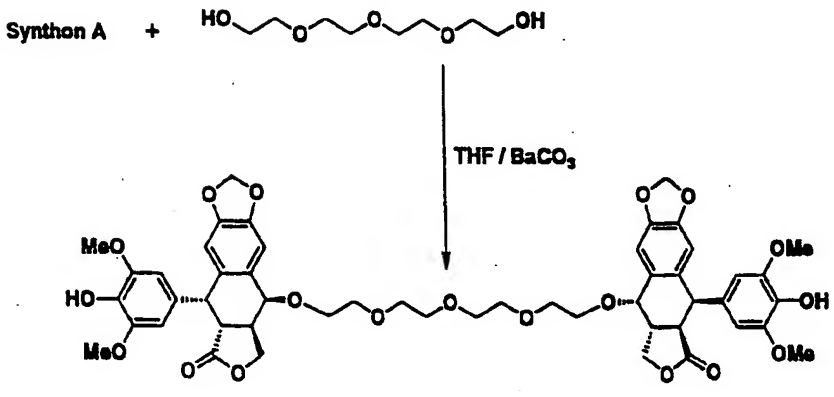
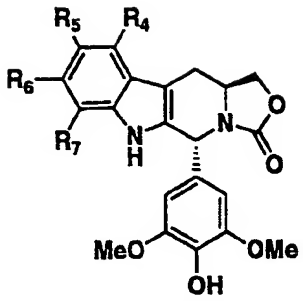


FIG. 16



R_4=R_5=R_6=R_7=H - Azatoxin

R_4=R_6=R_7=H; R_5=OH - Synthon D

R_4=R_6=R_5=H; R_7=OH - Synthon E

R_7=R_6=R_5=H; R_4=NH_2 - Synthon F

R_7=R_4=R_5=H; R_6=NH_2 - Synthon G

FIG. 13

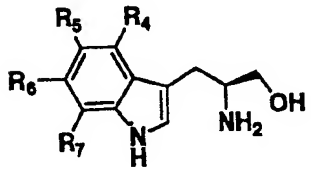


FIG. 14

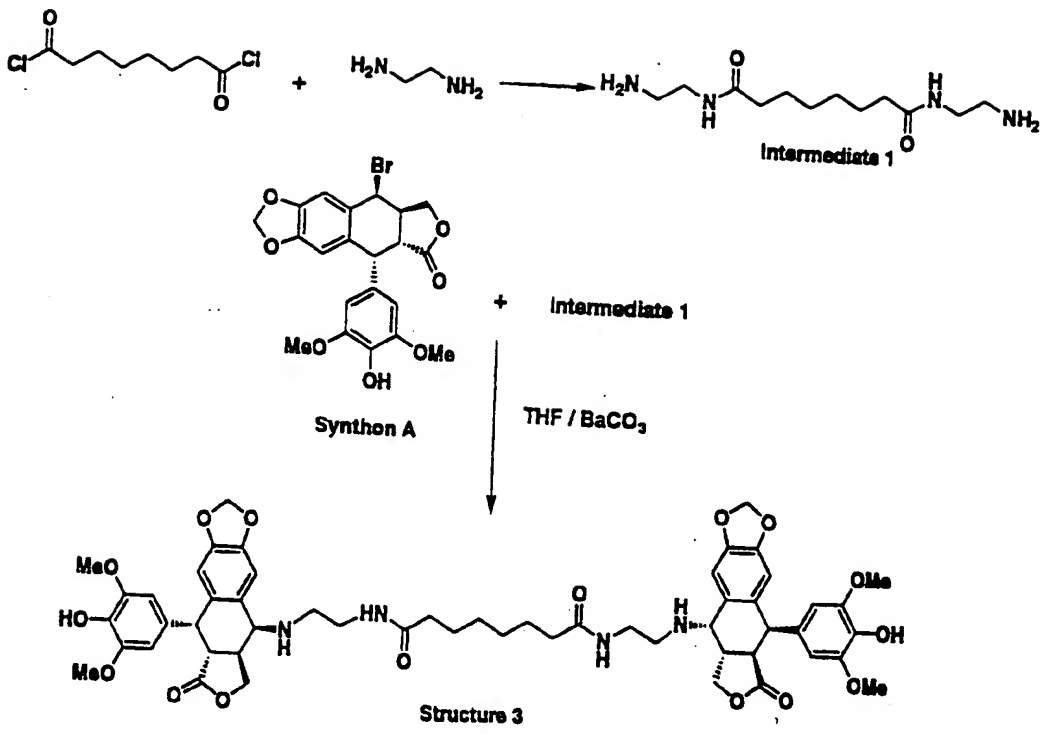

Intermediate 1
Br
Synthon A
+ Intermediate 1
THF / BaCO3
Structure 3

FIG. 17

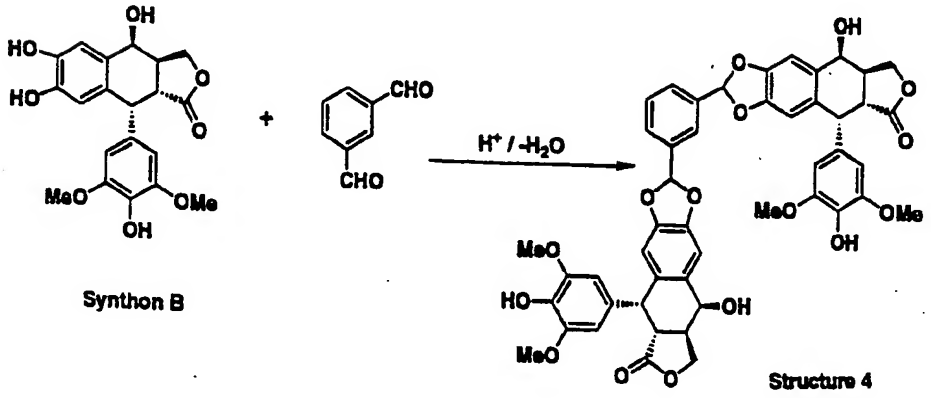
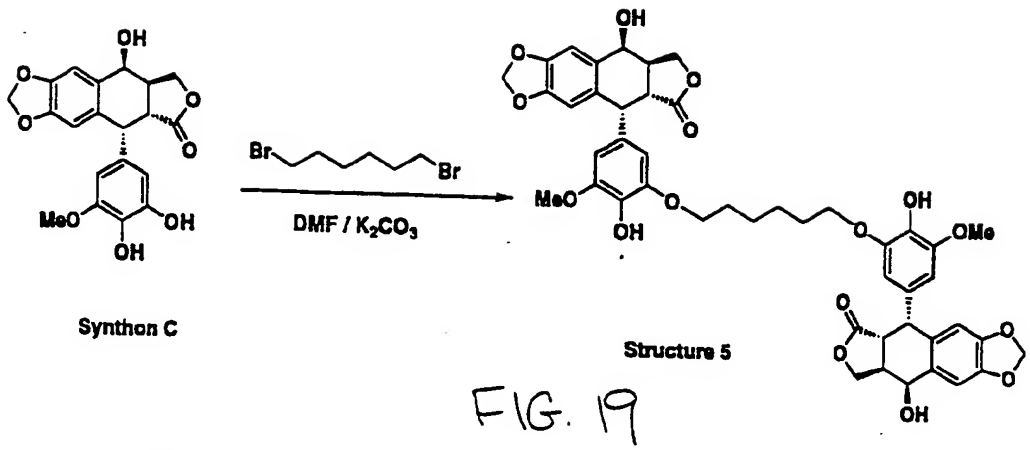

Synthon B
H+ / -H2O
Structure 4

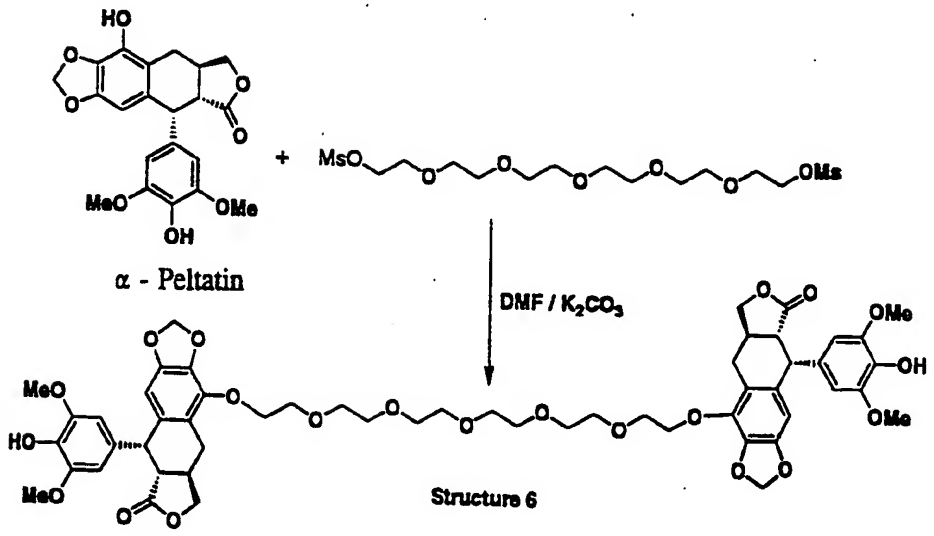
FIG. 18



Synthon C

Structure 5

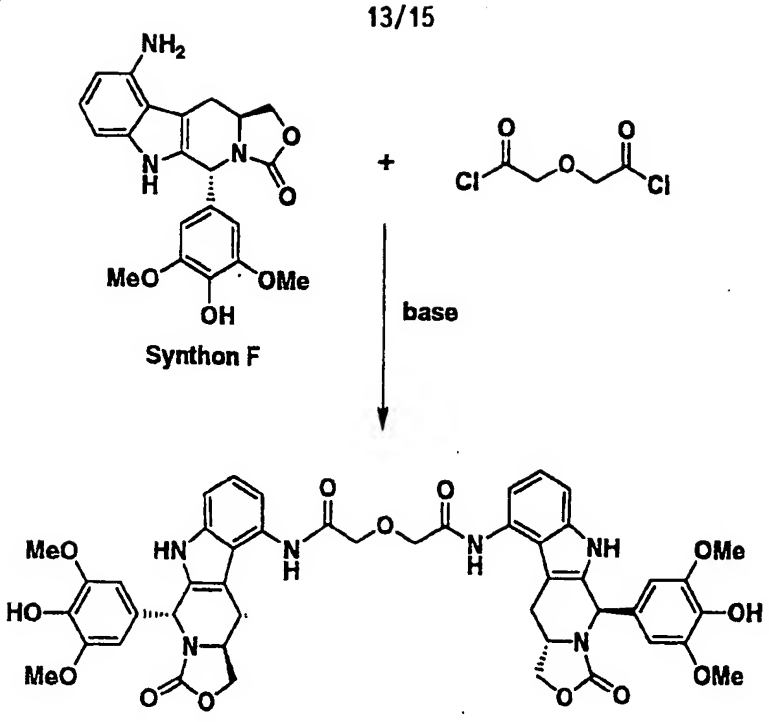
FIG. 19



α - Peltatin

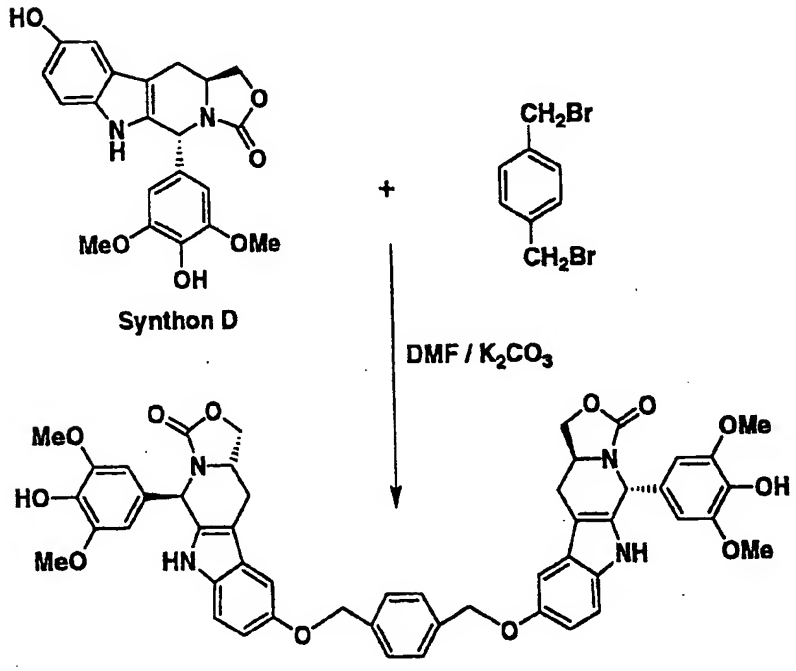
Structure 6

FIG. 20



Structure 7

FIG. 21



Structure 8

FIG 22

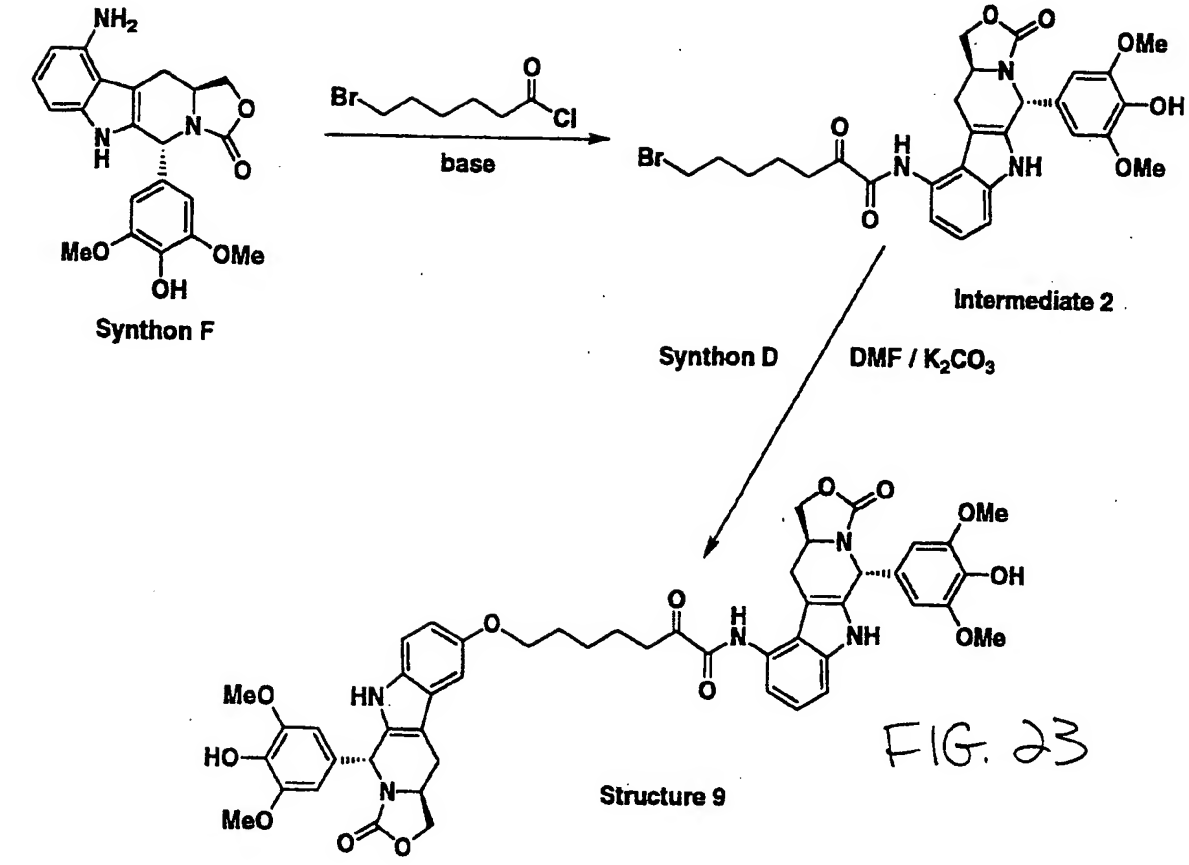

Synthon F
base
Intermediate 2
Synthon D
DMF / K_2CO_3
Structure 9

FIG. 23

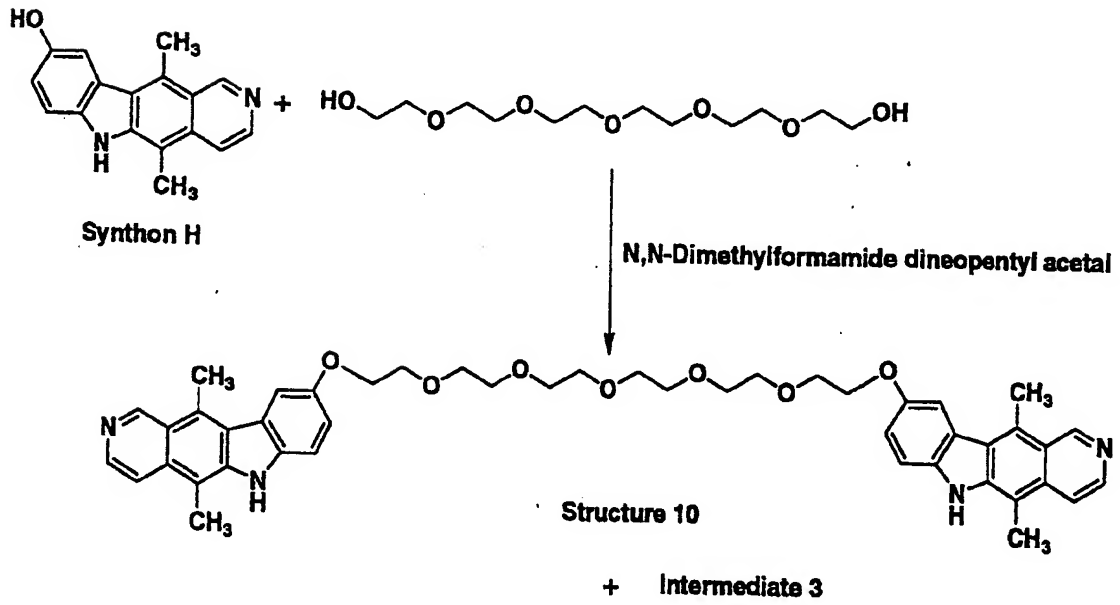

Synthon H
N,N-Dimethylformamide dineopentyl acetal
Structure 10
+ Intermediate 3

FIG. 24

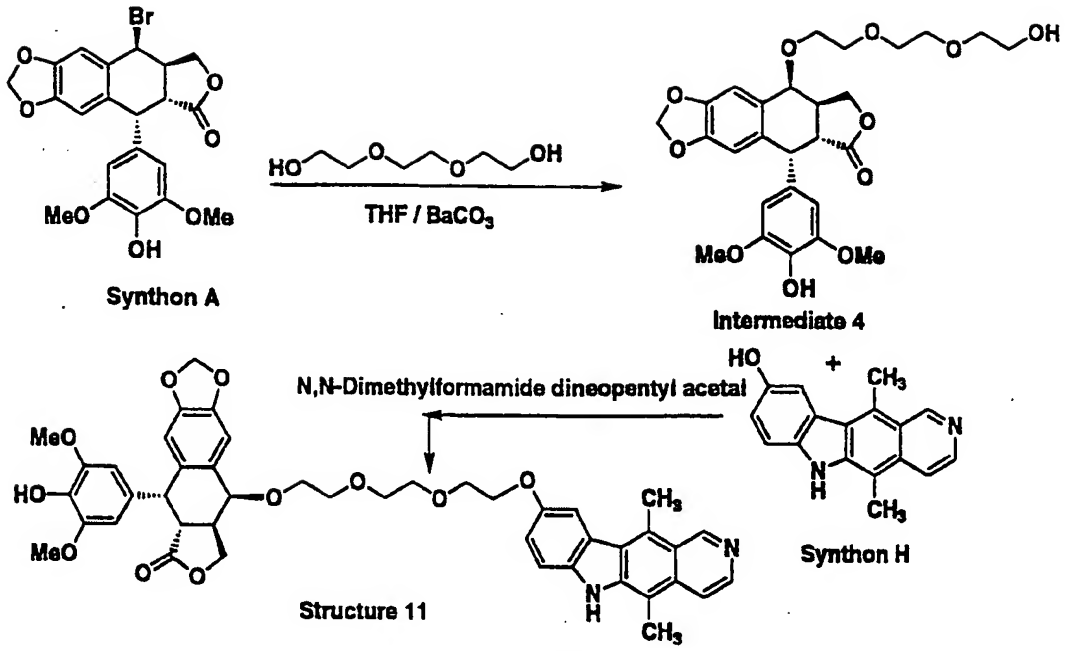

Br
HO
OH
THF / BaCO3
MeO
OMe
OH
Synthon A
Intermediate 4
N,N-Dimethylformamide dineopentyl acetal
HO
CH3
N
H
Synthon H
Structure 11

FIG 25

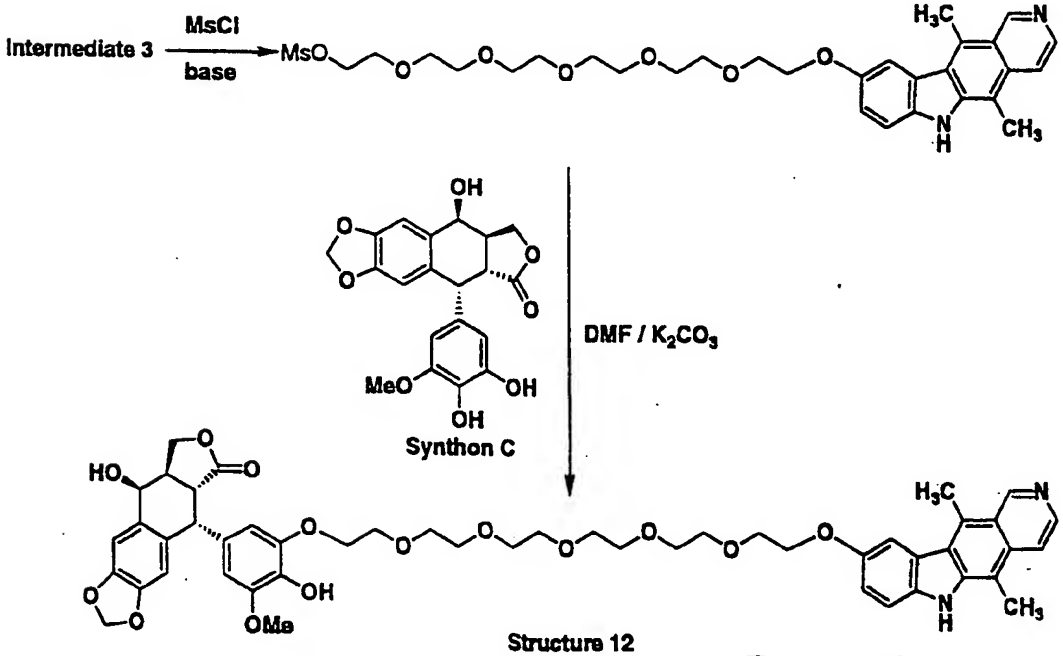

Intermediate 3
MsCl
base
MsO
H3C
N
CH3
DMF / K2CO3
Synthon C
Structure 12

FIG 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 536/16.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BROWN et al. 'Quinolones.' In: Antibiotic and Chemotherapy, 7th Ed. Editor: O'Grady, F. Churchill Livingstone:UK, 1997, pages 419-452. See entire document, especially page 419 2nd column 3rd paragraph and page 422-423 'Mode of Action'.	1-52
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16/04/92), see Abstract, page 3 lines 1-23, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 AUGUST 1999	Date of mailing of the international search report 18 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MAURIE E. GARCIA Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FAN et al. Self Assembly of a Quinobenzoxazine-Mg^{2+} Complex on DNA: A New Paradigm for the Structure of the Quinolone Bacterial Gyrase-DNA Complex. J. Med. Chem. 03 February 1995, Vol. 38, No. 3, pages 408-424, see entire article, especially pages 408-409 and 415-416.	1-52
Y	EHRHARDT et al. Structure-Activity Studies of Quinolone-Penems in Genetically Defined Strains of *Escherichia coli*. Antimicrobial Agents and Chemotherapy. November 1997, Vol. 41, No. 11, pages 2570-2572, see entire article.	1-52
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	26-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566; C07G 11/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

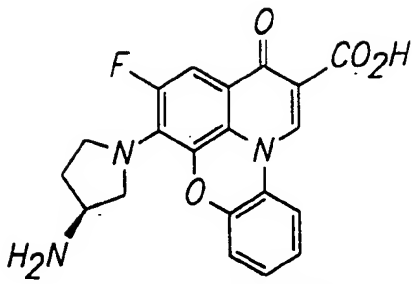
424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 536/16.8

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

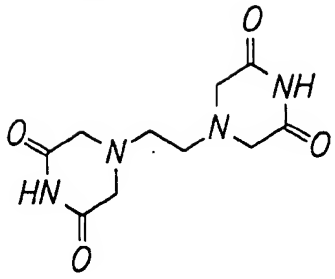
APS, STN (CAPLUS, BIOSIS, MEDLINE, SCISEARCH)
Search Terms: quinolone, chemical?, link?, multivalent, combinatorial, topoisomerase, bacterial, type II, inhibit?, antibiotic, antimicrobial, conjugate

51. The method according to Claim 50, wherein steps (e) and (f) are repeated from 2-50 times.

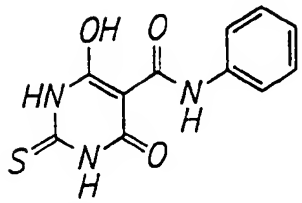
52. The method according to Claim 51, wherein steps (e) and (f) are repeated from 5-50 times.



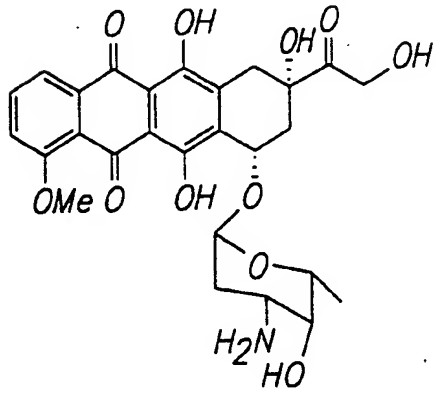
A-62176



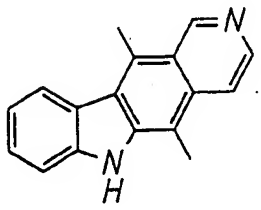
ICRF-154



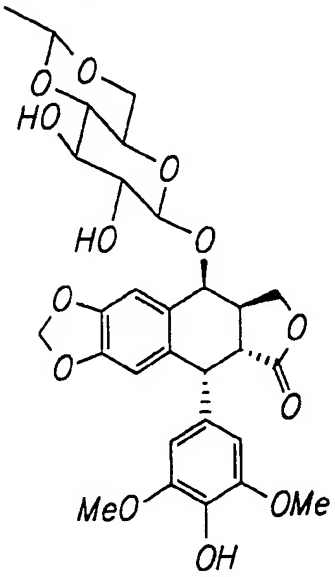
Merbarone



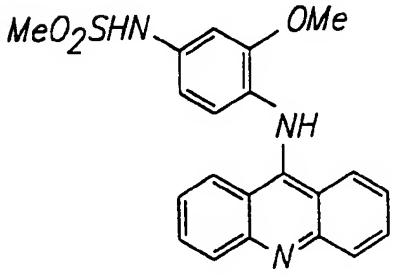
Doxorubicin



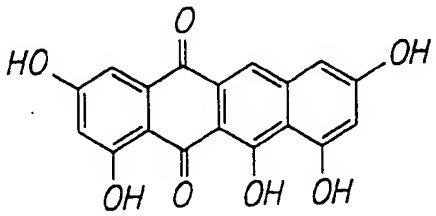
Ellipticine



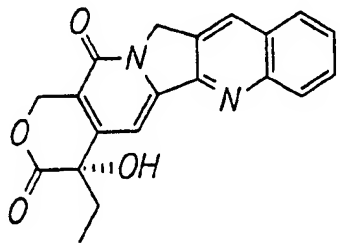
Etoposide



Amsacrine



Saintopin



Camptothecin

FIG. 1

FIG. 2

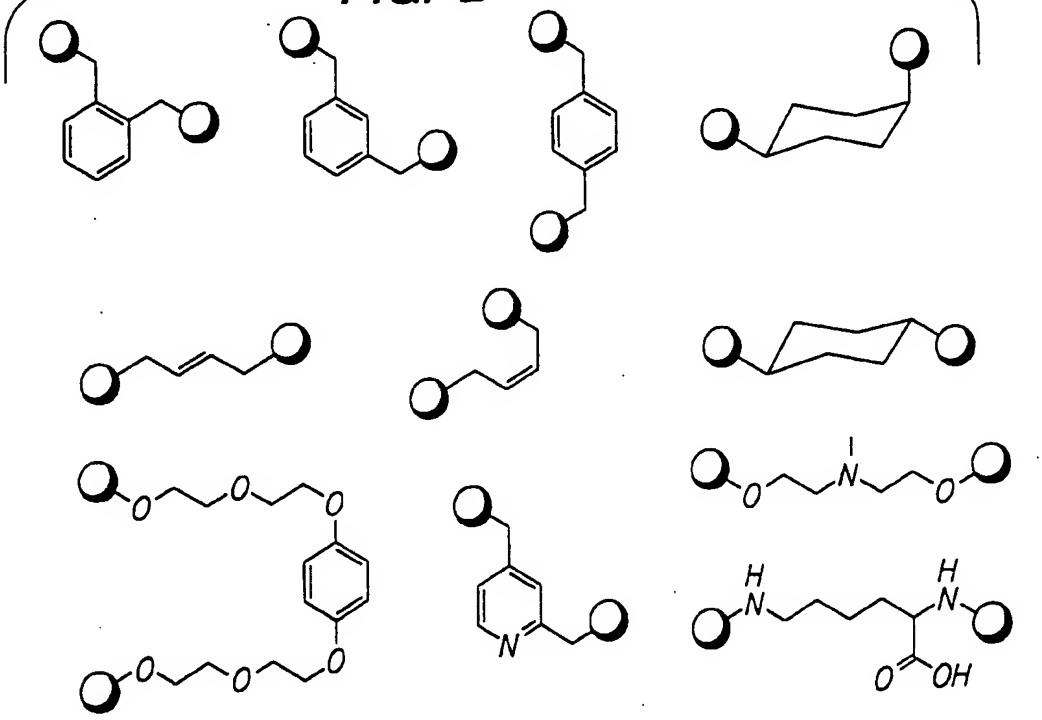


FIG. 3

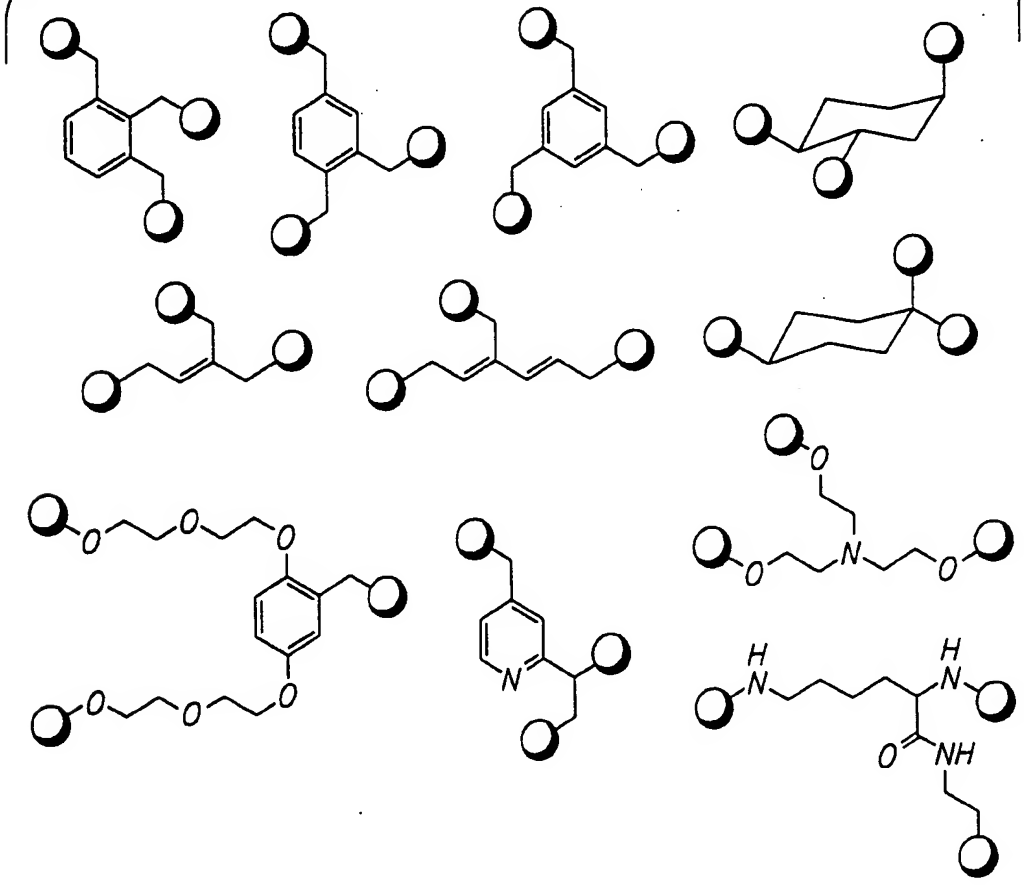


FIG. 4

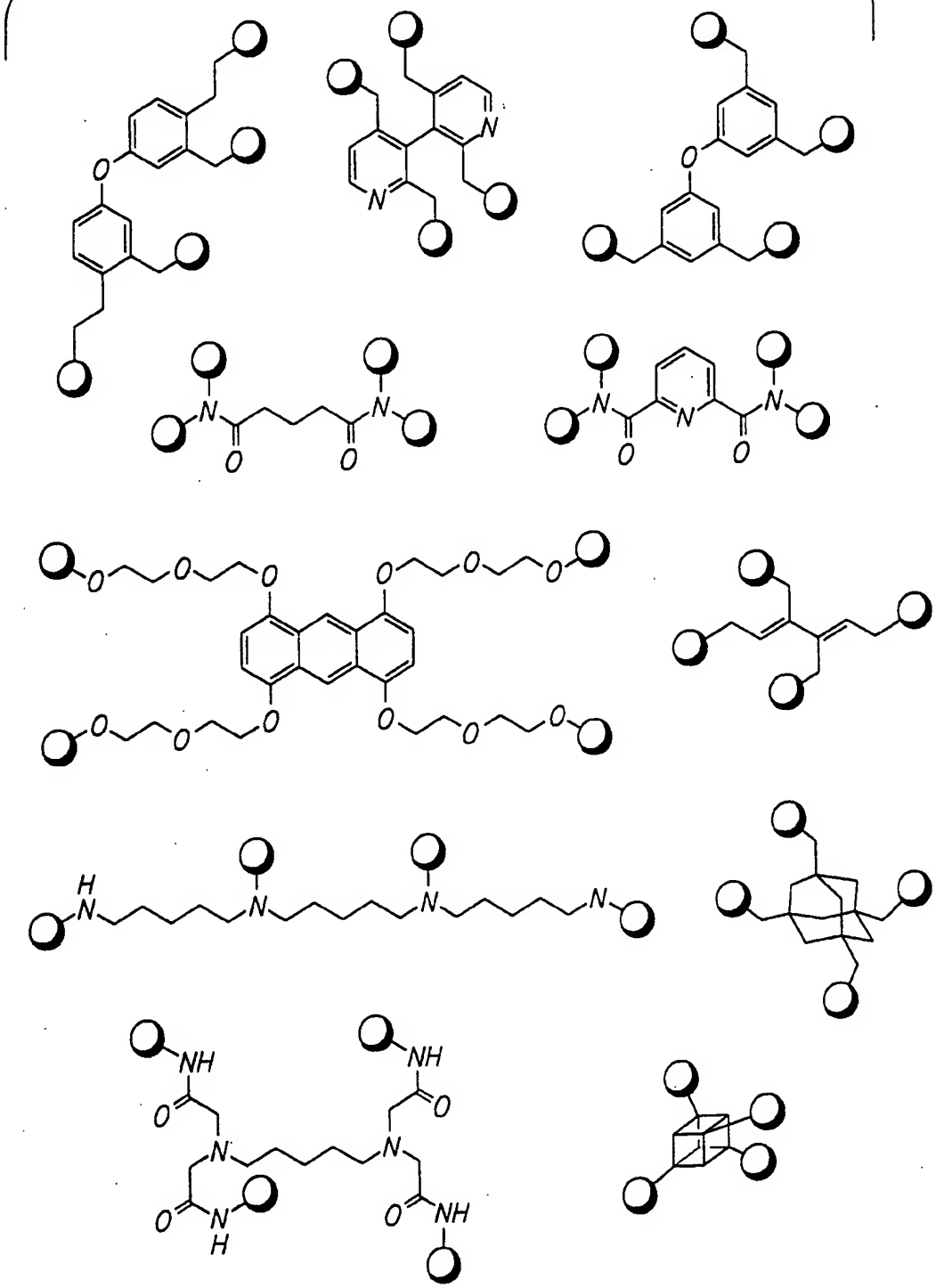
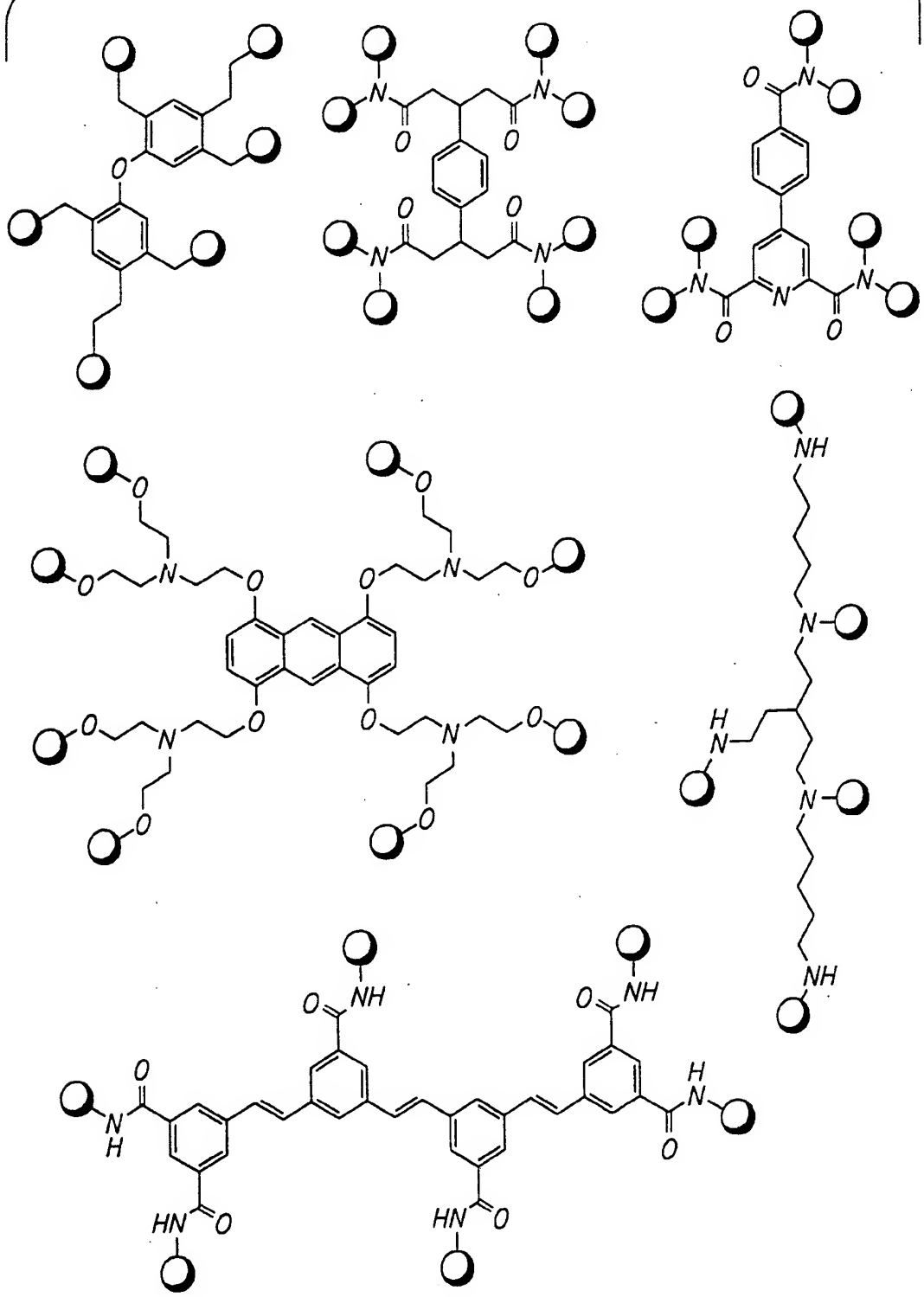


FIG. 5



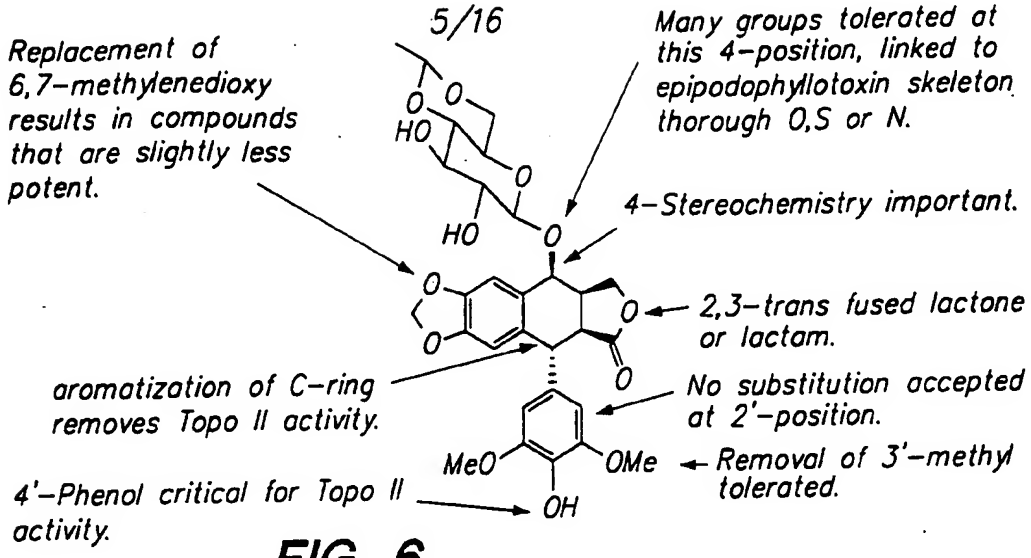
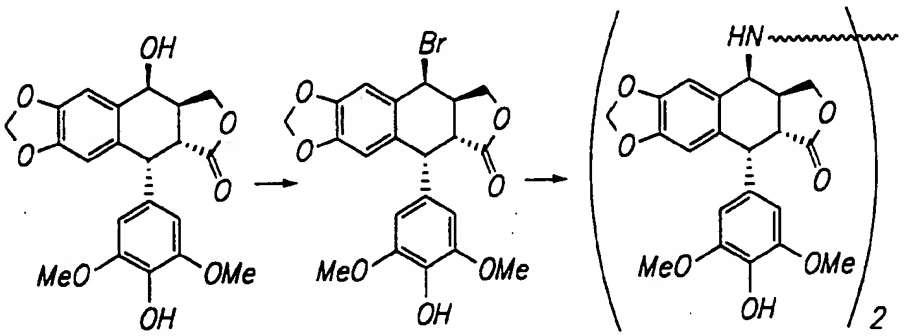
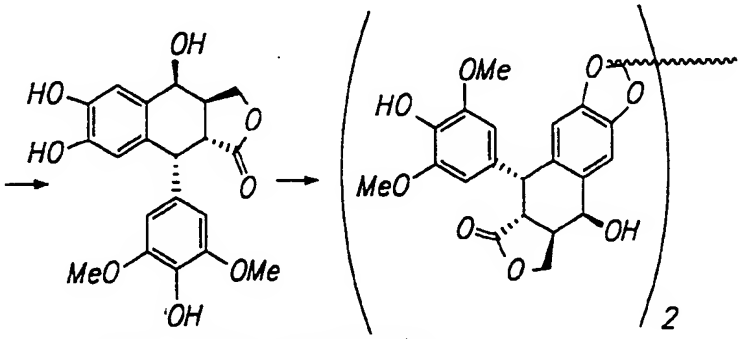

Replacement of 6,7-methylenedioxy results in compounds that are slightly less potent.
Many groups tolerated at this 4-position, linked to epipodophyllotoxin skeleton thorough O,S or N.
4-Stereochemistry important.
2,3-trans fused lactone or lactam.
aromatization of C-ring removes Topo II activity.
No substitution accepted at 2'-position.
Removal of 3'-methyl tolerated.
4'-Phenol critical for Topo II activity.

FIG. 6


4-position dimerisation


6,7-position dimerisation

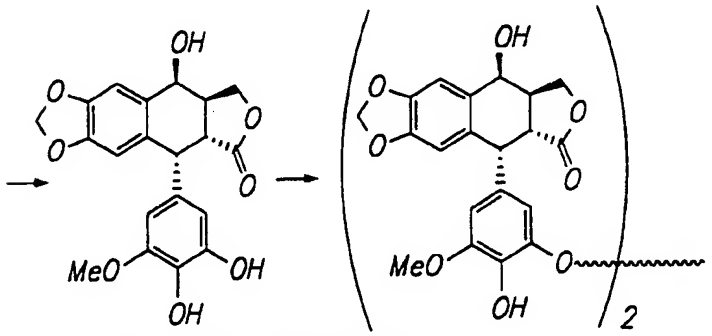

3'-position dimerisation

FIG. 7

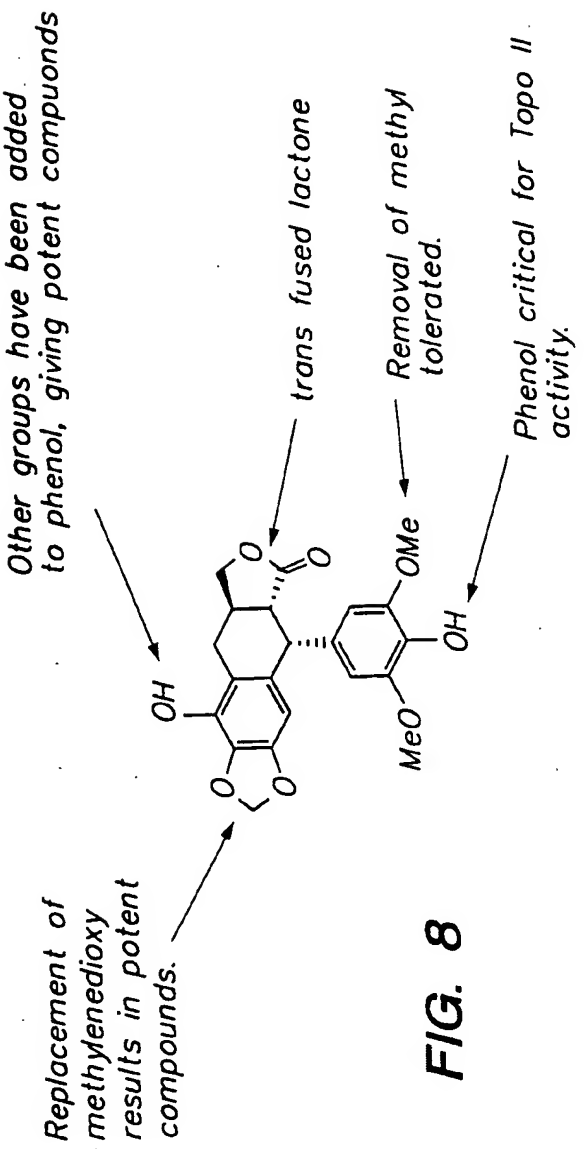

Other groups have been added to phenol, giving potent compounds
trans fused lactone
Removal of methyl tolerated.
Phenol critical for Topo II activity.
Replacement of methylenedioxy results in potent compounds.
OH
OMe
MeO
OH
O
O
O
O

FIG. 8

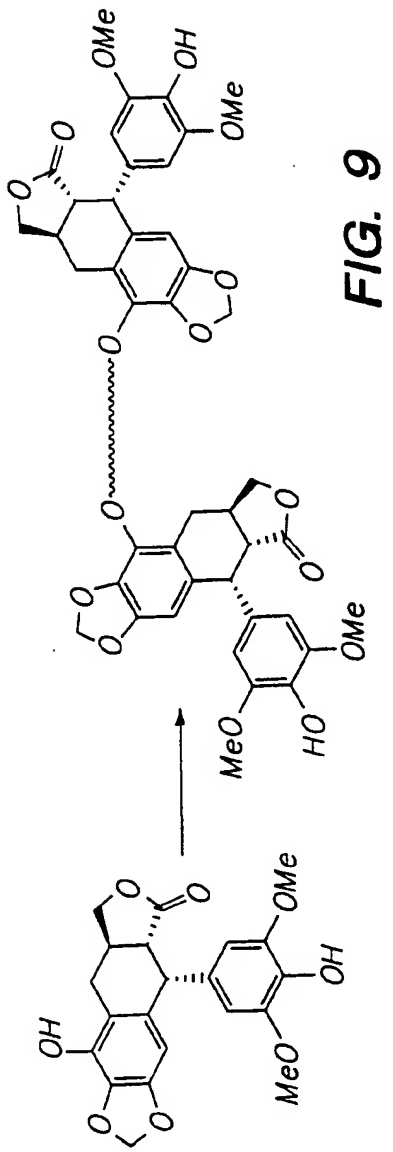

OMe
OH
OMe
O
O
O
O
O
O
O
O
O
O
OMe
MeO
HO
OH
OMe
MeO
O
O
O
O

FIG. 9

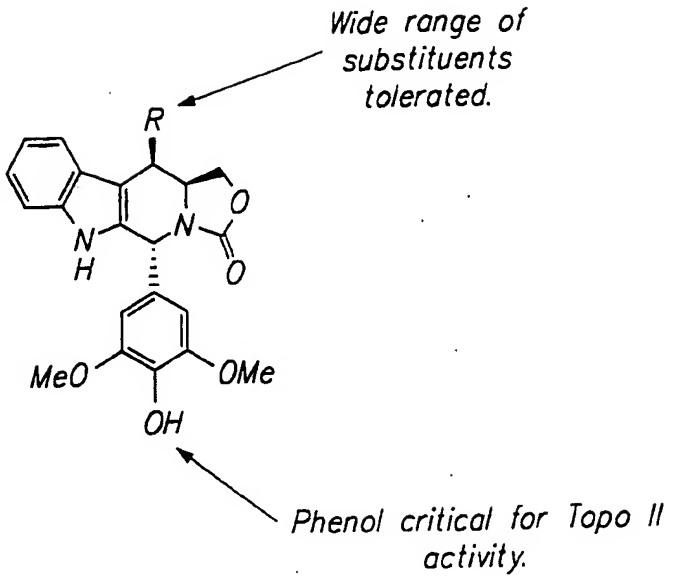

Wide range of substituents tolerated.
R
O
N
O
N
H
O
MeO
OMe
OH
Phenol critical for Topo II activity.

FIG. 10

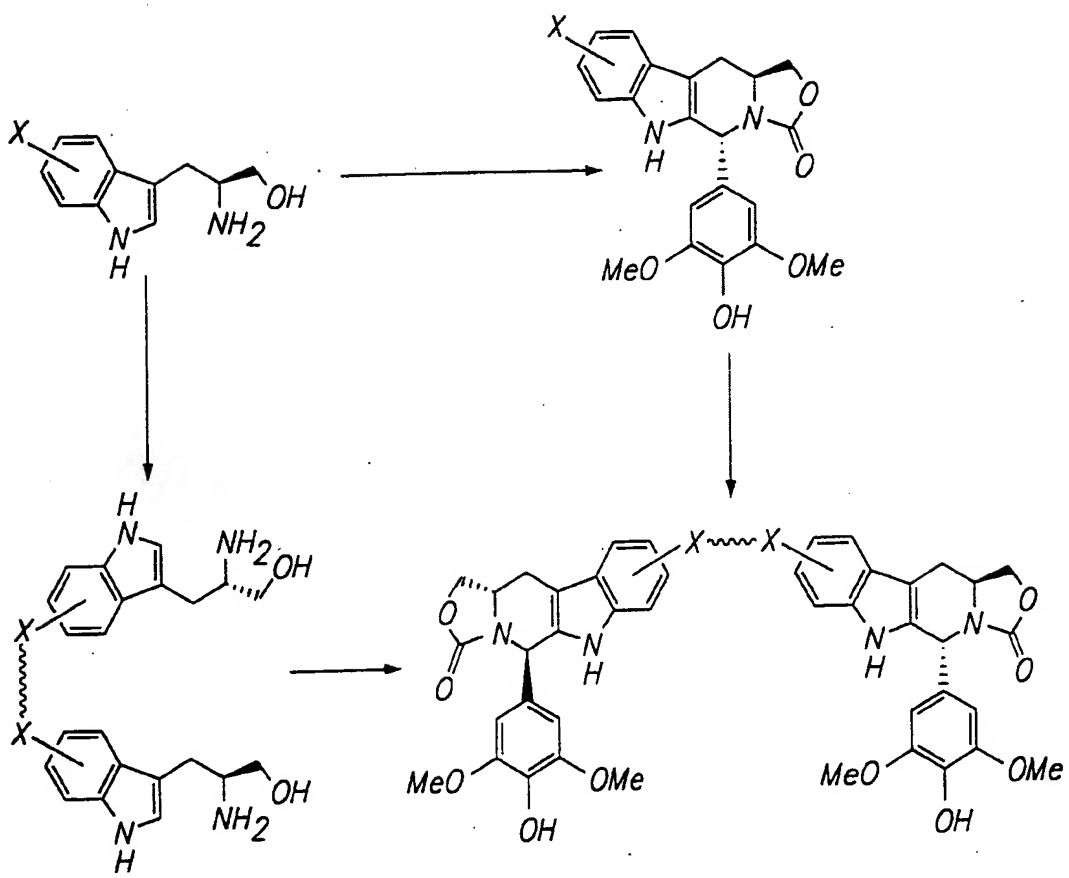

X
OH
NH2
N
H
MeO
OMe
OH
X
X

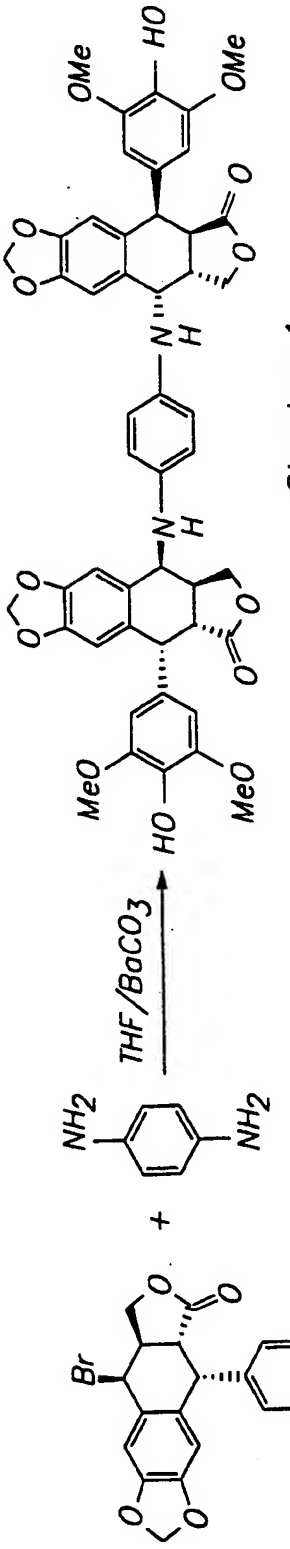
FIG. 11

FIG. 12

$R_4=R_5=R_6=R_7=H$ – Azatoxin
$R_4=R_6=R_7=H$; $R_5=OH$ – Synthon D
$R_4=R_6=R_5=H$; $R_7=OH$ – Synthon E
$R_7=R_6=R_5=H$; $R_4=NH_2$ – Synthon F
$R_7=R_4=R_5=H$; $R_6=NH_2$ – Synthon G

FIG. 13

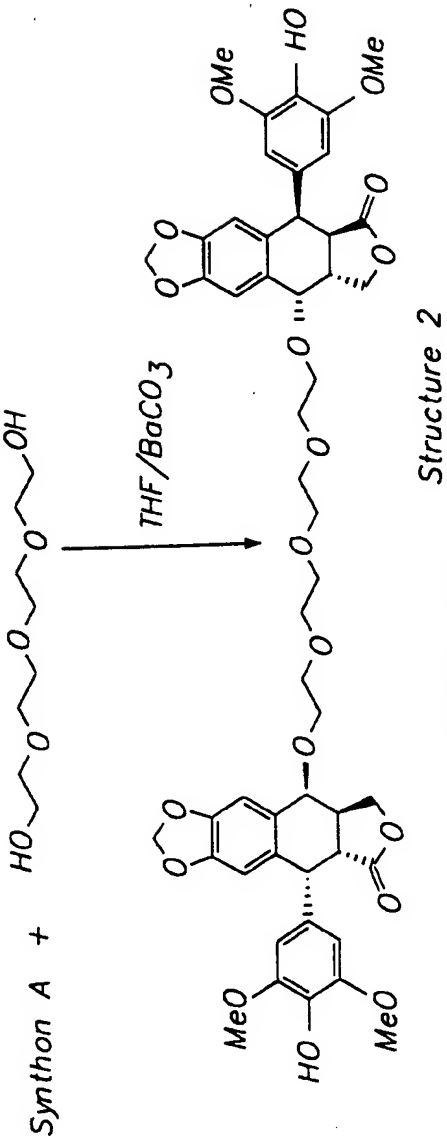
FIG. 14



Synthon A

Structure 1

FIG. 15



Synthon A

Structure 2

FIG. 16

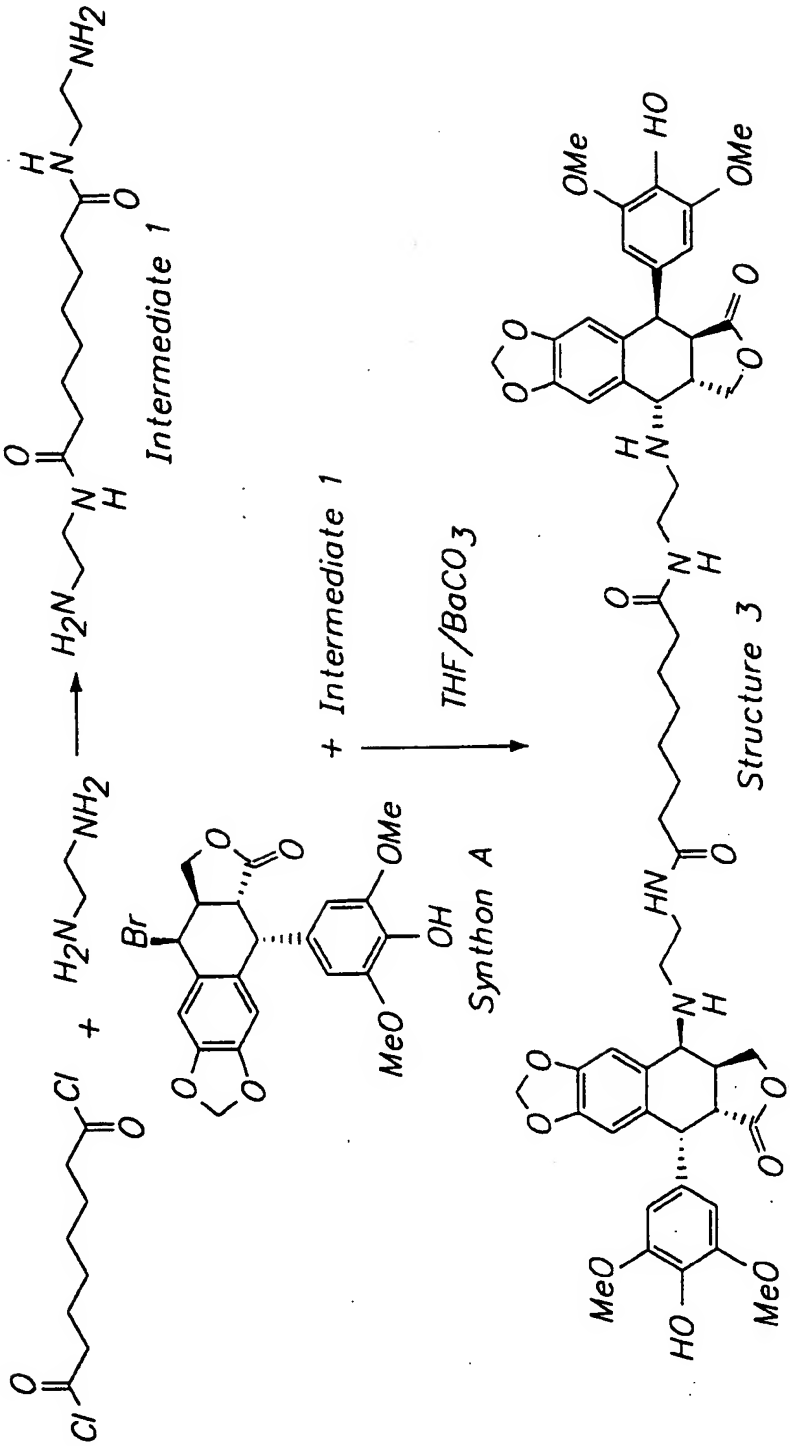

Intermediate 1
+ Intermediate 1
THF/BaCO3
Synthon A
Structure 3

FIG. 17

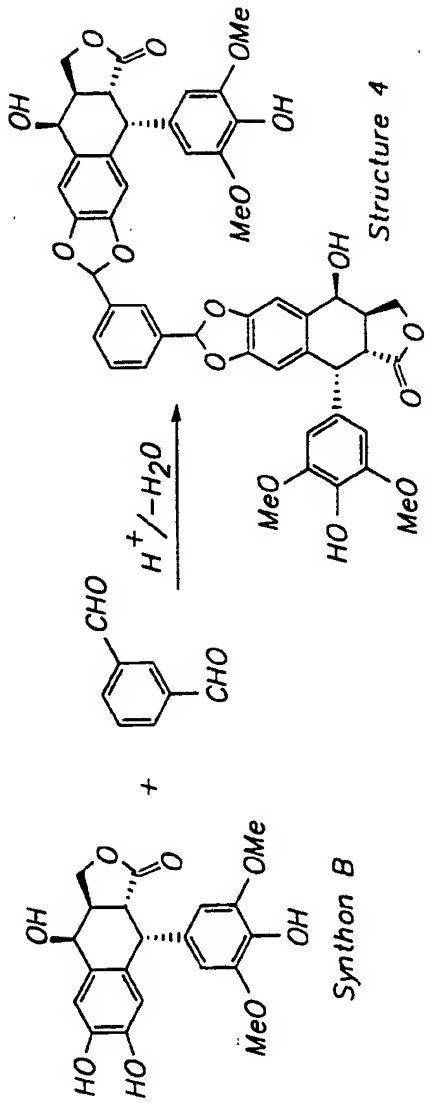

OMe
OH
MeO
OH
O
O
O
O
O
O
Structure 4
OH
O
O
MeO
HO
MeO
H+/-H2O
CHO
CHO
+
OMe
OH
MeO
HO
HO
Synthon B

FIG. 18

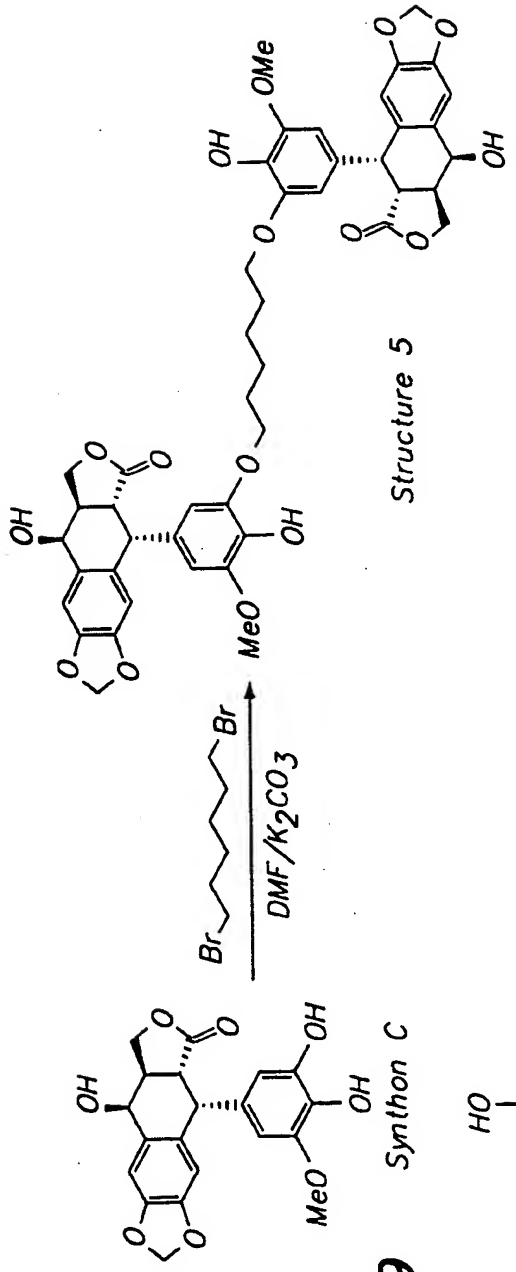

Br
Br
DMF/K2CO3
Structure 5
Synthon C

FIG. 19

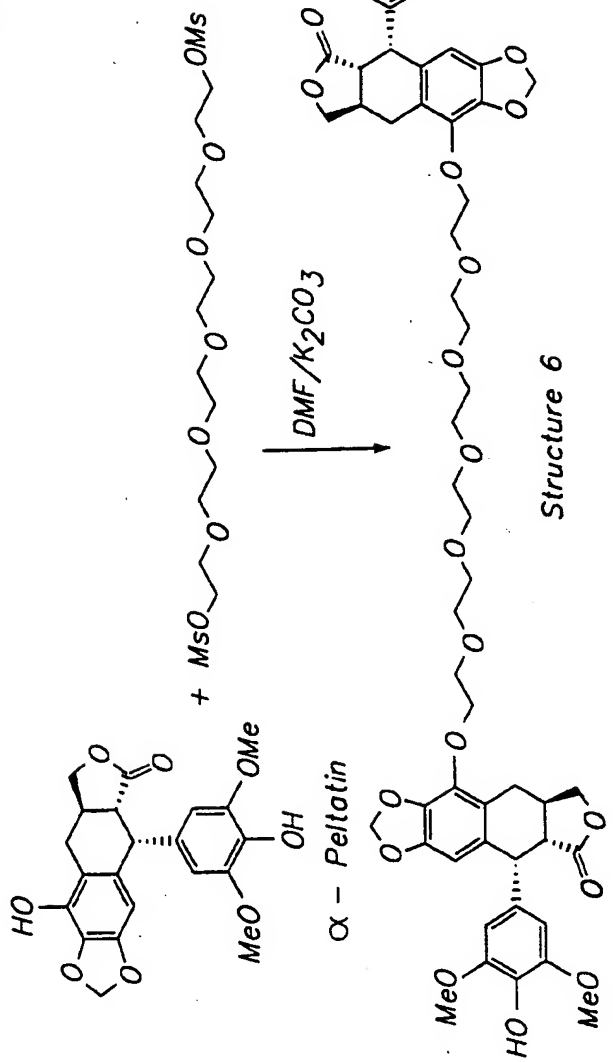

+ MsO
OMs
DMF/K2CO3
α - Peltatin
Structure 6

FIG. 20

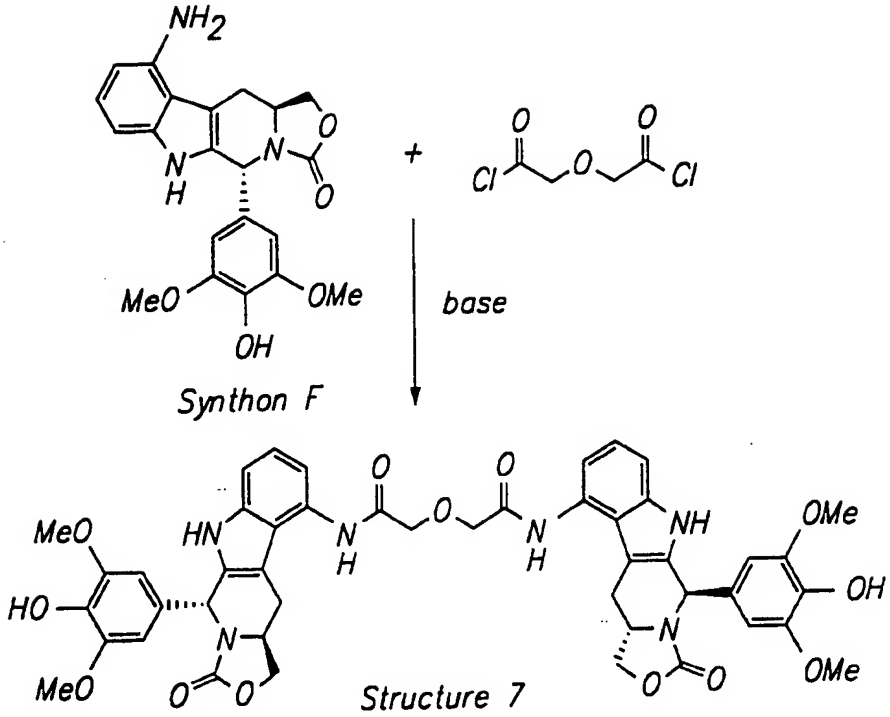

NH2
MeO
OMe
OH
Synthon F
base
Cl
Cl
MeO
HO
MeO
HN
NH
OMe
OH
OMe
Structure 7

FIG. 21

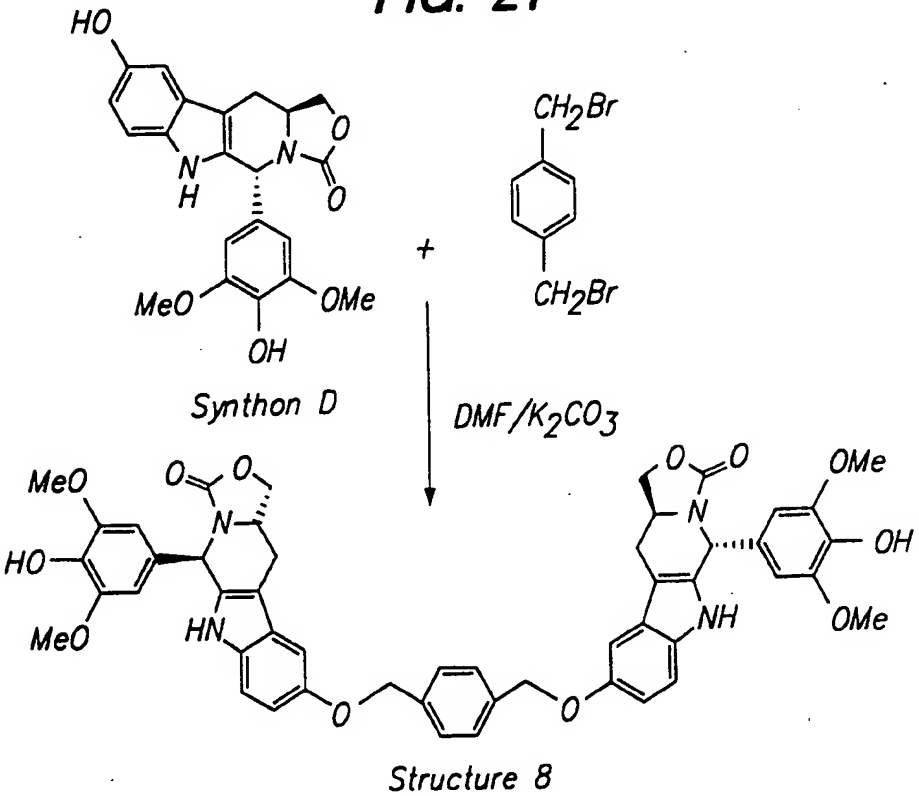

HO
MeO
OMe
OH
Synthon D
CH2Br
CH2Br
DMF/K2CO3
MeO
HO
MeO
HN
NH
OMe
OH
OMe
Structure 8

FIG. 22

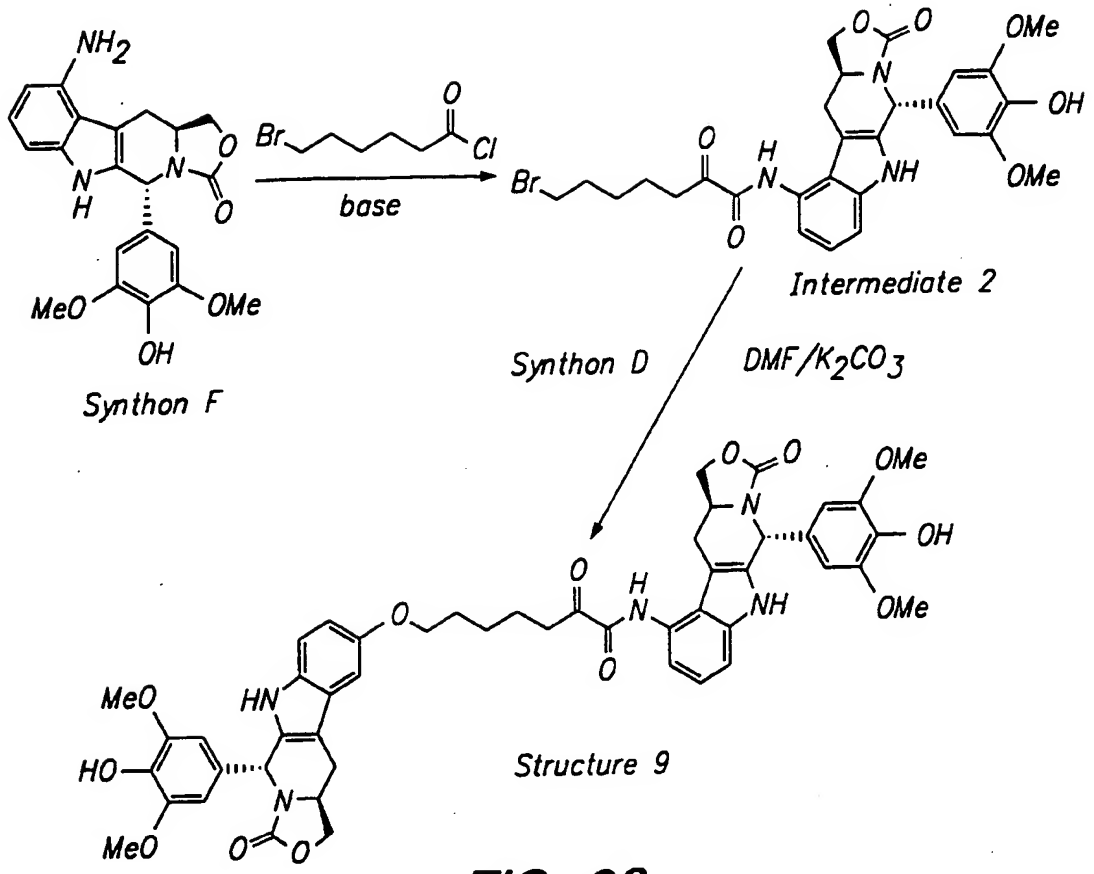

NH2
Br
Cl
base
Br
OMe
OH
OMe
Intermediate 2
Synthon F
MeO
OMe
OH
Synthon D
DMF/K2CO3
OMe
OH
OMe
MeO
HO
MeO
HN
Structure 9

FIG. 23

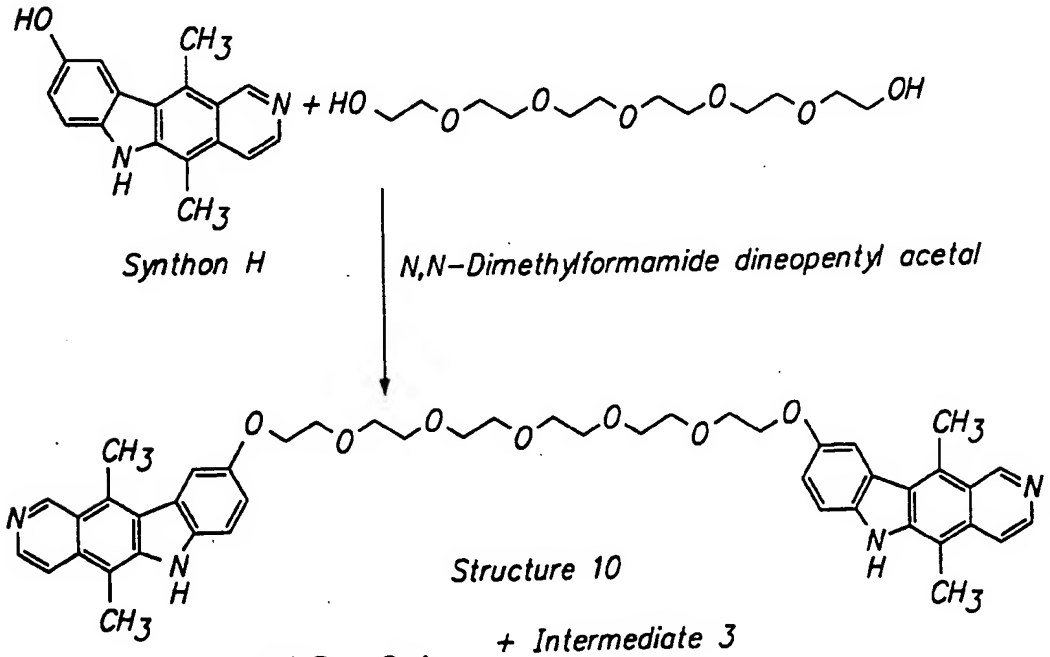

HO
CH3
N + HO
OH
Synthon H
N,N-Dimethylformamide dineopentyl acetal
CH3
CH3
CH3
Structure 10
+ Intermediate 3

FIG. 24

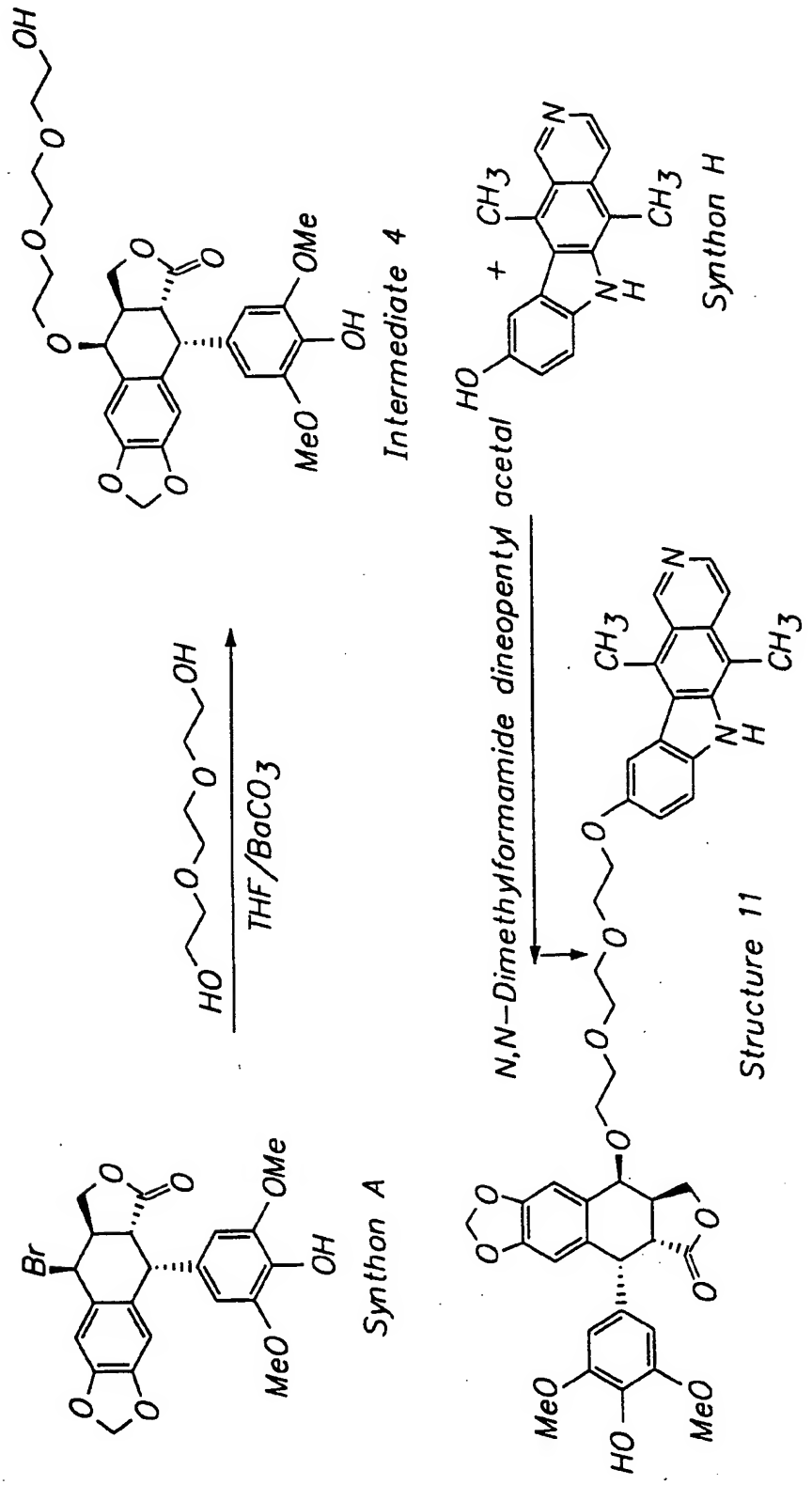

Intermediate 4
Synthon H
THF/BaCO3
Synthon A
N,N-Dimethylformamide dineopentyl acetal
Structure 11

FIG. 25

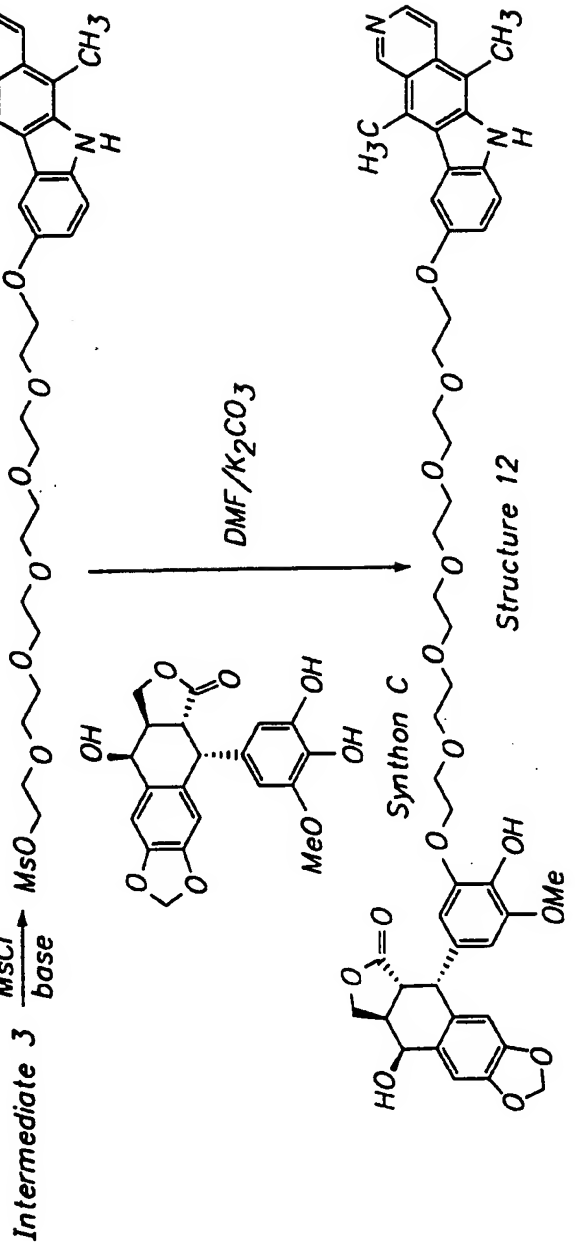

Intermediate 3
MsCl
base
MsO
DMF/K2CO3
Synthon C
Structure 12
H3C
CH3
N
H
OH
MeO
OMe
HO
O
=O

FIG. 26

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 536/16.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BROWN et al. 'Quinolones.' In: Antibiotic and Chemotherapy, 7th Ed. Editor: O'Grady, F. Churchill Livingstone:UK, 1997, pages 419-452. See entire document, especially page 419 2nd column 3rd paragraph and page 422-423 'Mode of Action'.	1-52
Y	WO 92/05802 A1 (NEORX CORPORATION) 16 April 1992 (16/04/92), see Abstract, page 3 lines 1-23, page 4 lines 20-27, page 5 lines 6-18, page 21 lines 4-33, page 22 lines 1-8 and claim 1.	1-52

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 11 AUGUST 1999	Date of mailing of the international search report 18 OCT 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MAURIE E. GARCIA Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	FAN et al. Self Assembly of a Quinobenzoxazine-Mg^{2+} Complex on DNA: A New Paradigm for the Structure of the Quinolone Bacterial Gyrase-DNA Complex. J. Med. Chem. 03 February 1995, Vol. 38, No. 3, pages 408-424, see entire article, especially pages 408-409 and 415-416.	1-52
Y	EHRHARDT et al. Structure-Activity Studies of Quinolone-Penems in Genetically Defined Strains of *Escherichia coli*. Antimicrobial Agents and Chemotherapy. November 1997, Vol. 41, No. 11, pages 2570-2572, see entire article.	1-52
Y	SHUKER et al. Discovering High-Affinity Ligands for Proteins: SAR by NMR. Science. 29 November 1996, Vol. 274, pages 1531-1534. See entire article, especially Figure 1.	26-52

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/12908

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 39/00, 39/44, 39/395, 51/00; C07K 2/00, 4/00; G01N 33/53, 33/543, 33/566; C07G 11/00

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/1.11, 9.1, 178.1, 193.1; 435/7.1, 7.2; 436/501, 518; 530/345, 389.1, 402, 807; 536/16.8

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, STN (CAPLUS, BIOSIS, MEDLINE, SCISEARCH)
Search Terms: quinolone, chemical?, link?, multivalent, combinatorial, topoisomerase, bacterial, type II, inhibit?, antibiotic, antimicrobial, conjugate